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(57) Abstract

An enzymatic nucleic acid molecule which cleaves RNA associated with development or maintenance of an architic condition, induction of graft tolerance or reversal of an immune response. In particular, the ribozyme sequences are directed to an mRNA encoding B7-1, B7-2, B7-3, CD40 and/or stromelysin. Also provided are ribozymes where the uracil in positions 4 and/or ? are substituted, as well as methods for the synthesis of 2'-alkylnucleotides, 2'-O-alkylthioalkyl, or 2'-alkylthioalkylnucleotides. The application further describes a method for diprotection of RNA with aqueous ethylamine, a method for synthesis of a basic ribonucleoside mimetics, and transcription units comprising an RNA polymerase II promoter, a U6 small nuclear promoter, or an adenovirus VA1 promoter system.

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METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND REVERSAL OF IMMUNE RESPONSES

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Background of the Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

In one aspect, this invention relates to methods for inhibition of osteoarthritis, in particular, inhibition of genetic expression which leads to a reduction or elimination of extracellular matrix digestion by matrix metalloproteinases.

There are several types of arthritis, with osteoarthritis and rheumatoid arthritis being predominar.t. Osteoarthritis is a slowly progressive disease characterized by degeneration of articular cartilage with proliferation and remodeling of subchondral bone. It presents with a clinical picture of pain, deformity, and loss of joint motion. Rheumatoid arthritis is a chronic systemic inflammatory disease. Rheumatoid arthritis may be mild and relapsing or severe and progressive, leading to joint deformity and incapacitation.

Arthritis is the major contributor to functional impairment among the older population. It is the major cause of disability and accounts for a large proportion of the hospitalizations and health care expenditures of the elderly. Arthritis is estimated to be the principal cause of total incapacitation for about one million persons aged 55 and older and is thought to be an important contributing cause for about one million more.

Estimating the incidence of osteoarthritis is difficult for several reasons. First, osteoarthritis is diagnosed objectively on the basis of reading radiographs, but many people with radiologic evidence of disease have no obvious symptoms. Second, the estimates of prevalence are based upon clinical evaluations because radiographic data is not available for all afflicted joints. In the NHANESI survey of 1989, data were based upon a thorough musculoskeletal evaluation during which any abnormalities of the spine, knee,

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hips, and peripheral joints were noted as well as other specific diagnoses. Based on these observations, 12% of the US population between 25 and 74 years of age have osteoarthritis.

It is generally agreed that rheumatoid arthritis has a world-wide distribution and affects all racial and ethnic groups. The exact prevalence in the US is unknown but has been estimated to range between 0.5% and 1.5%. Rheumatoid arthritis occurs at all age levels and generally increases in prevalence with advancing age. It is 2-3 times more prevalent in women than in men and peak incidence occurs between 40-60 years of age. In addition to immunological factors, environmental, occupational and psychosocial factors have been studied for potential etiologic roles in the disease.

The extracellular matrix of multicellular organisms plays an important role in the formation and maintenance of tissues. The meshwork of the extracellular matrix is deposited by resident cells and provides a framework for cell adhesion and migration, as well as a permeability barrier in cell-cell communication. Connective tissue turnover during normal growth and development or under pathological conditions is thought to be mediated by a family of neutral metalloproteinases, which are zinc-containing enzymes that require calcium for full activity. The regulation of metalloproteinase expression is cell-type specific and may vary among species.

The best characterized of the matrix metalloproteinases, interstitial collagenase (MMP-1), is specific for collagen types I, II, and III. MMP-1 cleaves all three chains of the triple helix at a single point initiating sequential breakdown of the interstitial collagens. Interstitial collagenase activity has been observed in rheumatoid synovial cells as well as in the synovial fluid of patients with inflammatory arthritis. Gelatinases (MMP-2) represent a subgroup of the metalloproteinases consisting of two distinct gene products; a 70 kDa gelatinase expressed by most connective tissue cells, and a 92 kDa gelatinase expressed by inflammatory phagocytes and tumor cells. The larger enzyme is expressed by macrophages, SV-40 transformed fibroblasts, and neutrophils. The smaller enzyme is secreted by H-ras transformed bronchial epithelial cells and tumor cells, as well as normal human skin fibroblasts. These enzymes degrade gelatin (denatured collagen) as well as native

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collagen type XI. Stromelysin (MMP-3) has a wide spectrum of action on molecules composing the extracellular matrix. It digests proteoglycans, fibronectin, laminin, type IV and IX collagens and gelatin, and can remove the N-terminal propeptide region from procollagen, thus activating the collagenase. It has been found in human cartilage extracts, rheumatoid synovial cells, and in the synovium and chondrocytes of joints in rats with collagen-induced arthritis.

Both osteoarthritis and rheumatoid arthritis are treated mainly with compounds that inhibit cytokine or growth-factor induced synthesis of the matrix metalloproteinases which are involved in the extracellular matrix destruction observed in these diseases. Current clinical treatments rely upon dexamethasone and retinoid compounds, which are potent suppressors of a variety of metalloproteinases. The global effects of dexamethasone and retinoid treatment upon gene expression in treated cells make the development of alternative therapies desirable, especially for long termtreatments. Recently, it was shown that gamma-interferon suppressed lipopolysaccharide induced collagenase and stromelysin production in cultured macrophages. Also, tissue growth factor-β (TGF-β) has been shown to block epidermal growth factor (EGF) induction of stromelysin synthesis in vitro. Experimental protocols involving gene therapy approaches include the controlled expression of the metalloproteinase inhibitors TIMP-1 and TIMP-2. Of the latter three approaches, only y-interferon treatment is currently feasible in a clinical application.

Sullivan and Draper, International PCT Publication No. WO 94/02595 and Draper et al., International PCT Publication No. WO 95/13380 disclose the use of ribozymes to treat arthritis.

In a second aspect, the invention relates to methods for the induction of graft tolerance, treatment of autoimmune diseases, inflammatory disorders and allergies in particular, by inhibition of B7-1, B7-2, B7-3 and CD40.

An adaptive immune response requires activation, clonal expansion, and differentiation of a class of cells termed T lymphocytes (T cells). T cell activation is a multi-step process requiring several signalling events between

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the T cell and an antigen presenting cell. The ensuing discussion details signals that are exchanged between T cells and antigen presenting B cells. Similar pathways are thought to occur between T cells and other antigen presenting cells such as monocytes or follicular dendritic cells.

T cell activation is initiated when the T-cell receptor (TCR) binds to a specific antigen that is associated with the MHC proteins on the surface of an antigen presenting cell. This primary stimulus activates the T cell and induces expression of CD40 ligand (CD40L) on the surface of the T cell. CD40L then interacts with its cognate receptor, CD40, which is constitutively expressed on the surface of B cells; CD40 transduces the signal leading to B cell activation. 10 B cell activations result in the expression of B7-1, B7-2 and/or B7-3, which in turn interacts with constitutively expressed CD28 on the surface of T cells. The interaction generates a secondary co-stimulatory signal that is required to fully activate the T cell. Complete T cell activation via the T cell receptor and CD28 leads to cytokine secretion, clonal expansion, and differentiation. If the T cell receptor is engaged, absence of this secondary co-stimulus mediated by ... CD28, then the T cell is inactivated, either by clonal anergy (nonresponsiveness or reduced reactivity of the immune system to specific antigen(s)) or clonal deletion (Jenkins et al., 1987 Proc. Natl. Acad. Sci. USA 20 Thus, engagement of the TCR without a concommitant costimulatory signal results in a state of tolerance toward the specific antigen recognized by the T cell. This co-stimulatory signal can be mediated by the binding of B7-1 or B7-2 or B7-3, present on activated antigen-presenting cells, to CD28, a receptor that is constitutively expressed on the surface of the T cell (Marshall et al., 1993 J Clin Immun 13, 165-174; Linsley, et al., 1991 J Exp Med 173, 721; Koulova et al., 1991 J Exp Med 173, 759; Harding et al., 1992 Nature 356, 607).

Several homologs of B7 (now known as B7-1; Cohen, 1993 Science 262, 844) are expressed in activated B cells (Freeman et al., 1993 Science 262, 907; Lenschow et al., 1993 Proc Natl Acad Sci USA 90, 11054; Azuma et al., 1993 Nature 366, 76; Hathcock et al., 1993 Science 262, 905; Freeman et al., 1993 Science 262, 909). B7-1 and B7-3 are only expressed on the surface of a subset of B cells after 48 hours of contact with T cells. In contrast, B7-2 mRNA is constitutively expressed by unstimulated B cells and increases 4-fold

within 4 hours of activation (Freeman et al., 1993 Science 262, 909; Boussiotis et al., 1993 Proc Natl Acad Sci USA 90, 11059). Since T cells commit to either the anergy or the activation pathway within 12-24 hours of the initial TCR signal, it is thought that B7-2 is the molecule responsible for the primary costimulatory signal. B7-1 and B7-3 may provide a subsequent signal necessary for clonal expansion. Antibodies to B7-2 completely block T cell proliferation in a mixed lymphocyte reaction (Azuma et al., 1993 supra), supporting the central role of B7-2 in T cell activation. These experiments indicate that inhibition of B7-2 expression (for example with a ribozyme) would likely induce anergy. Similarly, inhibition of CD40 expression by a ribozyme would prevent B7-2 upregulation and could induce tolerance to specific antigens.

B7 (B7-1) is a 60 KD modified trans-membrane glycoprotein usually present on the surface of antigen presenting cells (APC). B7 has two ligands—CD28 and CTLA4. Interaction of B7-1 with CD28 and/or CTLA4 causes activation of T cell responses (Janeway and Bottomly, 1994 *Cell* 76, 275).

B7-2 is a 70 KD (34 KD unmodified) trans-membrane glycoprotein found on the surface of APCs. B7-2 encodes a 323 amino-acid protein which is 26 % identical to human B7-1 protein. Like B7-1, CD28 and CTLA4 are selectively bound by B7-2. B7-2, unlike B7-1, is expressed on the surface of unstimulated B cells (Freeman et al., 1993 *supra*).

CD40 is a 45-50 KD surface glycoprotein found on the surface of late pre-B cells in bone marrow, mature B cells, bone marrow-derived dendritic cells and follicular dendritic cells (Clark and Ledbetter, 1994 *Nature* 367, 425).

Successful organ transplantation currently requires suppression of the recipient's immune system in order to prevent graft rejection and maintain good graft function. The available therapies, including cyclosporin A, FK506 and various monoclonal antibodies, all have serious side effects (Caine, 1992 Transplantation Proceedings 24, 1260; Fuleihan et al., 1994 J. Clin. Invest. 93, 1315; Van Gool et al., 1994 Blood 83, 176). In addition, existing therapies result in general immune suppression, leaving the patient susceptible to a variety of opportunistic infections. The ability to induce a state of long-term,

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antigen-specific tolerance to the donor tissue would revolutionize the field of organ and tissue transplantation. Since organ graft rejection is mediated by T cell effector function, the goal is to block specifically the activation of the subset of T cells that recognize donor antigens. A limitation in the field of transplantation is the supply of donor organs (Nowak 1994 *Science* 266, 1148). The ability to induce donor-specific tolerance would substantially increase the chances of successful allographs, xenographs, thereby greatly increasing the donor pool.

Such transplantation includes grafting of tissues and/or organ ie., implantation or transplantation of tissue and/or organs, from the body of an individual to a different place within the same or different individual. Transplantation also involve grafting of tissues and/or organs from one area of the body to another. Transplantation of tissues and/or organs between genetically dissimilar animals of the same species is termed as allogeneic transplantation. Transplantation of animal organs into humans is termed xenotransplants (for a review see Nowak; 1994 *Science* 266, 1148).

One therapy currently being dever ped that has similar potential to induce antigen-specific tolerance is treatment with a CTLA4-Ig fusion protein. "CTLA4" is a homologue of CD28 that binds B7-1 and B7-2 with high affinity. The engineered, soluble fusion protein, CTLA4-Ig, binds B7-1, thereby blocking its interaction with CD28. The results of CTLA4-Ig treatment in animal studies are mixed. CTLA4-Ig treatment significantly enhanced survival rates and ameliorated the symptoms of graft-versus host disease in a murine bone marrow tranplant model (Blazer et al., 1994 Blood 83, 3815). CTLA4-Ig induced long-term (>110 days) donor-specific tolerance in pancreatic islet xenographs (Lenschow et al., 1992 Science 257, 789). Conversely, in another study CTLA4-Ig treatment delayed but did not ultimately prevent cardiac allograft rejection (Turka, et al., 1992 Proc Natl Acad Sci U S A 89, 11102). Mice immunized with sheep erythrocytes in the presence of CTLA4-Ig failed to mount a primary immune response (Linsley, et al., 1992 Science 257, 792). A secondary immunization did elicit some response, however, indicating incomplete tolerance. Interestingly, identical results were obtained when CTLA4-Ig was administered 2 days after primary immunization, leading the authors to conclude that CTLA4-Ig blocked amplification rather than initiation

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of the immune response. Since CTLA4-Ig has been shown to dissociate more rapidly from B7-2 compared with B7-1, this may explain the failure to induce long term tolerance in this model (Linsley et al., 1994 *Immunity* 1, 793).

CTLA4:Ig has recently been shown to ameliorate symptoms of spontaneous autoimmune disease in lupus-prone mice (Finck et al., 1994 Science 265, 1225).

Linsley et al., WO 92/00092 describe B7 antigen as a ligand for CD28 receptor on T cells. The application states that-

"The B7 antigen, or its fragments or derivatives are reacted with CD28 positive T cells to regulate T cell interactions with other cells..... B7 antigen or CD28 receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses."

De Boer and Conroy, WO 94/01547 describe the use of anti-B7 and anti-CD40 antibodies to treat allograft transplant rejection, graft versus host disease and rhematoid arthritis. The application states that—

"...anti-B7 and anti-CD40 antibodies...can be used to prevent or treat an antibodymediated or immune system disease in a patient."

Since signalling via CD40 precedes induction of B-7, blocking the CD40-CD40L interaction would also have the potential to produce tolerance. According to one report, simultaneous treatment of mice with antibodies to CD40L and sheep red blood cells produced antigen-specific tolerance for up to 3 weeks following cessation of treatment (Foy et al., 1993 J Exp Med 178, 1567). Anti-CD40L also produces antigen specific tolerance in a pancreatic islet transplant model (R. Noelle, personal communication). Targeted inhibition of CD40 expression in B cells in addition to B7 would therefore afford double protection against activation of T cells.

Therapeutic agents used to prevent rejection of a transplanted organ are all cytotoxic compounds or antibodies designed to suppress the cell-mediated immune system. The side effects of these agents are those of immunosuppression and infections. The primary approved agents are azathioprine, corticosteroids, cyclosporine; the antibodies are antilymphocyte or antithymocyte globulins. All of these are given to individuals who have been as closely matched as possible to their donors by both major and minor

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histocompatibility typing. Since the principal problem in transplantation is an antigenic mismatch and the resulting need for cytotoxic therapy, any therapeutic improvement which decreases the local immune response without general immunosuppression should capture the transplant market.

Cyclosporine: At the end of the 1970's and early 1980's the introduction of cyclosporine revolutionized the transplantation field. It is a potent immunosuppressant which can inhibit immunocompetent lymphocytes specifically and reversibly. Its primary mechanism of action appears to be inhibition of the production and release of interleukin-2 by T helper cells. In addition it also interferes with the release of interleukin-1 by macrophages, as well as proliferation of B lymphocytes. It was approved by the FDA in 1983 and by 1989 was almost universally given to transplant recipients. At first it was believed that the toxicity and side effects from cyclosporine were minimal and it was hailed as a "wonder drug." Numerous side effects have been 15 progressively cited, including the appearance of lymphomas, especially in the gastrointestinal tract; acute and chronic nephrotoxicity; hypertension; hepatotoxicity; hirsutism; anemia; neurotoxicity; endocrine and neurological complications; and gastrointestinal distress. It is now widely acknowledged that the non-specific side effects of the drug demand caution and close 20 monitoring of its use. One-year survival rates for cadaver kidney transplants treated with cyclosporine is 80%, much better than the 50-60% rates without the drug. The one-year survival is almost 90% for transplants with related donors and the use of cyclosporine.

Azathioprine: In addition to cyclosporine, azathioprine is used for transplant patients. Azathioprine is one of the mercaptopurine class of drugs and inhibits nucleic acid synthesis. Patients are maintained indefinitely on daily doses of 1mg/kg or less, with a dosage adjusted in accordance with the white cell count. The drug may cause depression of bone marrow elements and may cause jaundice.

30 Corticosteroids: Prednisone, used in almost all transplant recipients, is usually given in association with azathioprine and cyclosporine. The dosage must be regulated carefully so as so prevent complications such as infection, development of cushingoid features, and hypertension. Usually the initial

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maintenance prednisone dosage is 0.5 mg/kg/d. This dosage is usually further decreased in the outpatient clinic until maintenance levels of about 10 mg/d for adults are obtained. The exact site of action of corticosteroids on the immune response is not known.

Antithymoblast or antilymphocyte globulin (ALG) and antithymocyte globulin (ATG): These are important adjunctive immunosuppressants. They are effective, particularly in induction of immunosuppressive therapy and in the treatment of corticosteroid-resistant rejection. Both ALG and ATG can be made by immunizing horses, rabbits, or sheep; the main source is horses. Lymphocytes from human peripheral blood, spleen, lymph nodes, or thymus serve as the immunogen.

Tacrolimus: On April 13, 1994 the Food and Drug Administration approved another drug to help prevent the rejection of organ transplants. The drug, tacrolimus, was approved only for use in liver transplant patients. An alternative to cyclosporine, the macrolide immunosuppressant tacrolimus is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984. Tacrolimus, isolated from the fungus Streptomyces tsukubaensis, possesses immunodepressant properties similar to but more potent than cyclosporine. It inhibits both cell-mediated and humoral immune responses. cyclosporine, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomized investigations of tacrolimus vs. cyclosporine, particularly in renal transplantation. Despite these drawbacks, tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids. potential for reductional withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with the cyclosporine. This benefit may be enhanced by reduced incidence of infectious complications. hypertension and hypercholesterolemia reported by some investigators. In other respects, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporine.

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In addition to induction of graft tolerance, T cell anergy can be used to reverse autoimmune diseases. Autoimmune diseases represent a broad category of conditions. A few examples include insulin-dependent diabetes mellitus (IDDM), multiple schlerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis. These seemingly disparate diseases all share the common feature of inappropriate immune response to specific self-antigens. Finck et al. *supra* have reported that CTLA4lg treatment of mice blocked auto-antibody production in a mice model of SLE. In fact, this effect was observed even when the CTLA4lg treatment was initiated during the advanced stages of the disease, suggesting that the autoimmune response was a reversible process.

Chappel, WO 94/11011 describes methods to treat autoimmune diseases by inducing tolerance to cells, tissues and organs. The application states that—

"Cells genetically engineered with DNA encoding a plurality of antigens of a cell, tissue, or organ to which tolerance is to be induced. The cells are free of co-stimulatory antigens, such as B7 antigen. Such cells induce T-cell anergy against the proteins encoded by the DNA, and may be administered to a patient in order to prevent the onset of or to treat at autoimmune disease, or to induce tolerance to a tissue or organ prior to transplantation."

Allergic reactions represent an immediate hypersensitivity response to environmental antigens, typically mediated by IgE antibodies. The ability to induce antigen-specific tolerance provides a powerful avenue to alleviate allergies by exposure to the antigen in conjunction with down-regulation of B7-1, B7-2, B7-3 or CD40.

The specific roles of B7-1, B7-2 and B7-3 in T cell activation remains to be determined. Some studies suggest that their functions are essentially redundant (Hathcock et al 1994 *J Exp. Med.* 180, 631), or that the differences observed in the kinetics of expression might simply indicate that B7-2 is important in the initiation of the co-stimulatory signal, while B7-1 plays a role in the amplification of that signal. Other studies point to more specific functions. For example, Kuchroo et al., 1995 *Cell* 80, 707, have reported that blocking B7-1 expression may favor a Th2 response, while blocking B7-2 expression favors a Th1 response. These two helper T cell subpopulations play distinct roles in the immune response and inflammatory disease. Th1 cells are

strongly correlated with auto-immune disease. Allergic responses are typically triggered by Th2 response. Therefore, the decision to target B7-1, B7-2, CD40 or a combination of the above will depend to the particular disease application.

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Summary of the Invention

Applicant notes that the inhibition of collagenase and stromelysin production in the synovial membrane of joints can be accomplished using ribozymes and antisense molecules. Ribozyme treatment can be a partner to current treatments which primarily target immune cells reacting to pre-existing tissue damage. Early ribozyme or antisense treatment which reduces the collagenase or stromelysin-induced damage can be followed by treatment with the anti-inflammatories or retinoids, if necessary. In this manner, expression of the proteinases can be controlled at both transcriptional and translational levels. Ribozyme or antisense treatment can be given to patients expressing radiological signs of osteoarthritis prior to the expression of clinical symptoms. Ribozyme or antisense treatment can impact the expression of stromelysin without introducing the non-specific effects upon gene expression which accompany treatment with the retinoids and dexamethasone. The ability of stromelysin to activate procollagenase indicates that a ribozyme or antisense molecule which reduces stromelysin expression can also be used in the treatment of both osteoarthritis (which is primarily a stromelysinassociated pathology) and rheumatoid arthritis (which is primarily related to enhanced collagenase activity).

25 While a number of cytokines and growth factors induce metalloproteinase activities during wound healing and tissue injury of a preosteoarthritic condition, these molecules are not preferred targets for therapeutic intervention. Primary emphasis is placed upon inhibiting the molecules which are responsible for the disruption of the extracellular matrix, because most people will be presenting radiologic or clinical symptoms prior to treatment. The most versatile of the metalloproteinases (the molecule which can do the most structural damage to the extracellular matrix, if not regulated)

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is stromelysin. Additionally, this molecule can activate procollagenase, which in turn causes further damage to the collagen backbone of the extracellular matrix. Under normal conditions, the conversion of prostromelysin to active stromelysin is regulated by the presence of inhibitors called TIMPs (tissue inhibitors of MMP). Because the level of TIMP in synovial cells exceeds the level of prostromelysin and stromelysin activity is generally absent from the synovial fluid associated with non-arthritic tissues, the toxic effects of inhibiting stromelysin activity in non-target cells should be negligible.

Thus, the invention features use of specific ribozyme molecules to treat or prevent arthritis, particularly osteoarthritis, by inhibiting the synthesis of the prostromelysin molecule in synovial cells, or by inhibition of other matrix metalloproteinases discussed above. Cleavage of targeted mRNAs (stromelysin mRNAs, including stromelysin 1, 2, and 3, and collagenase) expressed in macrophages, neutrophils and synovial cells represses the synthesis of the zymogen form of stromelysin, prostromelysin.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide bard sequence specific manner. It is said that such enzymatic RNA molecules can be targeted to virtually any RNA transcript and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Nat. Acad. of Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acid Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary basepairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct

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synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified mRNA target, and also has an enzymatic activity which is active to specifically cleave that mRNA. That is, the enzymatic RNA molecule is able to intermolecularly cleave mRNA and thereby inactivate a target mRNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. For *in vivo* treatment, complementarity between 30 and 45 bases is preferred; although lower numbers are also useful.

By "complementary" is meant a nucleotide sequence that can form hydrogen bond(s) with other nucleotide sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of base-paired interactions.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or basesubstitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89. 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

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In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA. Examples of such hammerhead motifs are described by Rossi et al., 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel et al., EPA 0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel et al., 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada et al., 1983 Cell 35, 849, Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target stromelysin encoding mRNAs such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However,

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these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol. 66, 1432-41; Weerasinghe et al., 1991 J. Virol. 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res. 23, 2259). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target mRNA encoding factors that contribute to disease pathology. Thus, ribozymes that cleave stromelysin mRNAs may represent novel therapeutics for the treatment of asthma.

Thus, in a first aspect, the invention features ribozymes that inhibit stromelysin production. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target stromelysin encoding mRNAs, preventing translation and stromelysin protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "inhibit" is meant that the activity or level of stromelysin encoding mRNAs and protein is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

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Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of stromelysin activity in a cell or tissue. By "related" is meant that the inhibition of stromelysin mRNAs and thus reduction in the level of stromelysin activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues <u>ex vivo</u>, or <u>in vivo</u> through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables All, AllI, AlV, AVI, AVIII and AIX. Examples of such ribozymes are shown in Tables AV, AVII, AVIII and AIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In a related aspect the invention features ribozymes that cleave target molecules and inhibit stromelysin activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

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By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

This class of chemicals exhibits a high degree of specificity for cleavage of the intended target mRNA. Consequently, the ribozyme agent will only affect cells expressing that particular gene, and will not be toxic to normal tissues.

The invention can be used to treat or prevent (prophylactically) osteoarthritis or other pathological conditions which are mediated by metalloproteinase activation. The preferred administration protocol is *in vivo* administration to reduce the level of stromelysin activity.

Thus, the invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of an arthritic condition, e.g., mRNA encoding stromelysin, and in particular, those mRNA targets disclosed in the accompanying tables, which include both hammerhead and hairpin target sites. In each case the site is flanked by regions to which appropriate substrate binding arms can be synthesized and an appropriate hammerhead or hairpin motif can be added to provide enzymatic activity, by methods described herein and known in the art. For example, referring to Figure 1, arms I and III are modified to be specific substrate-binding arms, and arm II remains essentially as shown.

Ribozymes that cleave stromelysin mRNAs represent a novel therapeutic approach to arthritic disorders like osteoarthritis. The invention features use of ribozymes to treat osteoarthritis, e.g., by inhibiting the synthesis of prostromelysin molecule in synovial cells or by the inhibition of matrix metalloproteinases. Applicant indicates that ribozymes are able to inhibit the secretion of stromelysin and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave stromelysin encoding mRNAs may be readily designed and are within the invention.

In other related aspects, the invention features a mammalian cell which includes an enzymatic RNA molecule as described above. Preferably, the

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mammalian cell is a human cell; and the invention features an expression vector which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the vector, <u>e.g.</u>, in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell; or a method for treatment of a disease or condition by administering to a patient an enzymatic RNA molecule as described above.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of an arthritic condition. Such enzymatic RNA molecules can be delivered exogenously or endogenously to infected cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The enzymatic RNA molecules of this invention can be used to treat arthritic or prearthritic conditions. Such treatment can also be extended to other related genes in nonhuman primates. Affected animals can be treated at the time of arthritic risk detection, or in a prophylactic manner. This timing of treatment will reduce the chance of further arthritic damage.

In another aspect, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (Cook et al., U.S. Patent 5,359,051)] and methods for their use to induce graft tolerance, to treat autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and to treatment of allergies.

In a preferred embodiment, the invention features use of one or more of the nucleic acid-based techniques to induce graft tolerance by inhibiting the synthesis of B7-1, B7-2, B7-3 and CD40 proteins.

Those in the art will recognize the other potential targets, for e.g., ICAM-1, VCAM-1, β 1 integrin (VLA4) are also suitable for treatment with the nucleic acid-based techniques described in the present invention.

By "inhibit" is meant that the activity of B7-1, B7-2, B7-3 and/or CD40 or level of mRNAs encoded by B7-1, B7-2, B7-3 and/or CD40 is reduced below that observed in the absence of the nucleic acid. In one embodiment, inhibition with ribozymes preferably is below that level observed in the presence of an enzymatically inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "equivalent" RNA to B7-1, B7-2, B7-3 and/or CD40 is meant to include those naturally occurring RNA molecules associated with graft rejection in various animals, including human, mice, rats, rabbits, primates and pigs.

By "antisense nucleic acid" is meant a non-enzymatic nucleic acid molecule that binds to another RNA (target RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 Nature 365, 566) interactions and alters the activity of the target RNA (for a review see Stein and Cheng, 1993 Science 261, 1004).

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Triple-helix formation has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 *Proc. Natl. Acad. Sci.USA* 89, 504).

By "gene" is meant a nucleic acid that encodes an RNA.

25 Ribozymes that cleave the specified sites in B7-1. B7-2, B7-3 and/or CD40 mRNAs represent a novel therapeutic approach to induce graft tolerance and treat autoimmune diseases, allergies and other inflammatory conditions. Applicant indicates that ribozymes are able to inhibit the activity of B7-1, B7-2, B7-3 and/or CD40 and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these

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sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs may be readily designed and are within the invention.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding B7-1, B7-2, B7-3 and/or CD40 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of B7-1, B7-2, B7-3 and/or CD40 activity in a cell or tissue. By "related" is meant that the inhibition of B7-1, B7-2, B7-3 and/or CD40 mRNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables BII, BIV, BVI, BVII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes are shown in Tables BIII, BV, BVI, BVII, BIX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit B7-1, B7-2, B7-3 and/or CD40 activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-

associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

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Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be \geq 2 base-pairs long.

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, Nature, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, Nature, 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, Nucl. Acids. Res., 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (i.e., n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases

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(preferably 3 - 20 bases, i.e., m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (i.e., r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (e.g., 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete basepairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (i.e., o and p is each independently from 0 to any number, e.g., 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases. " - " refers to a chemical bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNaseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of bodylabeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 shows in vitro cleavage of stromelysin mRNA by HH ribozymes.

Figure 8 shows inhibition of stromelysin expression by 21HH ribozyme in HS-27 fibroblast cell line.

Figure 9 shows inhibition of stromelysin expression by 463HH ribozyme in HS-27 fibroblast cell line.

Figure 10 shows inhibition of stromelysin expression by 1049HH ribozyme in HS-27 fibroblast cell line.

Figure 11 shows inhibition of stromelysin expression by 1366HH ribozyme in HS-27 fibroblast cell line.

Figure 12 shows inhibition of stromelysin expression by 1410HH ribozyme in HS-27 fibroblast cell line.

Figure 13 shows inhibition of stromelysin expression by 1489HH ribozyme in HS-27 fibroblast cell line.

Figure 14 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 15 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 16 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 17 shows the effect of phosphorothicate substitutions on the catalytic activity of 1049 2'-C-allyl HH ribozyme. A) diagrammatic representation of 1049 hammerhead ribozyme-substrate complex. 1049 U4-C-allyl P=S ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U₄) contains 2'-C-allyl substitution. Additionally, five nucleotides within the ribozyme, at the 5' and 3' termini, contain

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phosphorothicate substitutions. B) shows the ability of ribozyme described in Fig. 17A to decrease the level of stromelysin RNA in rabbit knee.

Figure 18 is a diagrammatic representation of chemically modified ribozymes targeted against stromelysin RNA. 1049 2'-amino P=S Ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 29 nucleotides contain 2'-O-methyl substitutions and two nucleotides (U₄ and U₇) contain 2'-amino substitution. Additionally, the 3' end of this ribozyme contains a 3'-3' linked inverted T and four nucleotides at the 5' termini contain phosphorothioate substitutions. Arrow-head indicates the site of RNA cleavage (site 1049). 1363 2'-Amino P=S, Human and Rabbit 1366 2'-Amino P=S ribozymes are identical to the 1049 2'-amino P=S ribozyme except that they are targeted to sites 1363 and 1366 within stromelysin RNAs.

Figure 19 shows 1049 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 20 shows 1363 2'-amino P=S ribozyme-mediated reduction in the level of Enomelysin mRNA in rabbit knee.

Figure 21 shows 1366 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figures 22a-d are diagrammatic representations of non-limiting examples of base modifications for adenine, guanine, cytosine and uracil, respectively.

Figure 23 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel et al., Nucleic Acids Res. 1992, 20:3252) showing specific substitutions in the catalytic core and substrate binding arms. Compounds 4, 9, 13, 17, 22 and 23 are described in Fig. 24.

Figure 24 is a diagrammatic representation of various nucleotides that can be substituted in the catalytic core of a hammerhead ribozyme.

Figure 25 is a diagrammatic representation of the synthesis of a 30 ribothymidine phosphoramidite.

Figure 26 is a diagrammatic representation of the synthesis of a 5-methylcytidine phosphoramidite.

Figure 27 is a diagrammatic representation of the synthesis of 5-bromouridine phosphoramidite.

Figure 28 is a diagrammatic representation of the synthesis of 6-azauridine phosphoramidite.

Figure 29 is a diagrammatic representation of the synthesis of 2,6-diaminopurine phosphoramidite.

Figure 30 is a diagrammatic representation of the synthesis of a 6-methyluridine phosphoramidite.

Figure 31 is a representation of a hammerhead ribozyme targeted to site A (HH-A). Site of 6-methyl U substitution is indicated.

Figure 32 shows RNA cleavage reaction catalyzed by HH-A ribozyme containing 6-methyl U-substitution (6-methyl-U4). U4, represents a HH-A ribozyme containing no 6-methyl-U substitution.

Figure 33 is a representation of a hammerhead ribozyme targeted to site B (HH-B). Sites of 6-methyl U substitution are indicated.

Figure 34 shows RNA cleavage reaction catalyzed by HH-B ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-B ribozyme containing no 6-methyl-U substitution.

Figure 35 is a representation of a hammerhead ribozyme targeted to site C (HH-C). Sites of 6-methyl U substitution are indicated.

Figure 36 shows RNA cleavage reaction catalyzed by HH-C ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-C ribozyme containing no 6-methyl-U substitution.

Figure 37 shows 6-methyl-U-substituted HH-A ribozyme-mediated inhibition of rat smooth muscle cell proliferation.

Figure 38 shows 6-methyl-U-substituted HH-C ribozyme-mediated inhibition of stromelysin protein production in human synovial fibroblast cells.

Figure 39 is a diagrammatic representation of the synthesis of pyridin-2-one nucleoside and pyridin-4-one nucleoside phosphoramidite.

- Figure 40 is a diagrammatic representation of the synthesis of 2-*O-t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl-b-D-ribofuranose phosphoramidite.
- Figure 41 is a diagrammatic representation of the synthesis of pseudouridine, 2,4,6-trimethoxy benzene nucleoside and 3-methyluridine phosphoramidite.
 - Figure 42 is a diagrammatic representation of the synthesis of dihydrouridine phosphoramidite.
- Figure 43 A) is diagrammatic representation of a hammerhead ribozyme targeted to site. B) shows RNA cleavage reaction catalyzed by hammerhead ribozyme with modified base substitutions at either position 4 or position 7.
- Figure 44 shows further kinetic characterization of RNA cleavage reactions catalyzed by HH-B ribozyme (A); HH-B with pyridin-4-one substitution at position 7 (B); and HH-B with phenyl substitution at position 7 (C).
 - Figure 45 is a diagrammatic representation of the synthesis of 2-O-t-Butyldimethylsilyl-5-O-Dimethoxytrityl-3-O-(2-Cyanoethyl-N,N-diisopropylphosphoramidite)-1-Deoxy-1-Naphthyl- β -D-Ribofuranose.
- Figure 46 is a diagrammatic representation of the synthesis of Synthesis of 2-O-t-Butyldimethylsilyl-5-O-Dimethoxytrityl-3-O-(2-Cyanoethyl-N,N-diisopropylphosphoramidite)-1-Deoxy-1-(p-Aminophenyl)-β-D-Ribofuranose.

Figure 47 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel et al. Nucleic Acids Res. 1992, 20, 3252) showing specific substitutions.

Figure 48 shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 49 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 50 is a diagrammatic representation of the synthesis of 2'-C-10 methylene and 2'-C-difluoromethylene uridine.

Figure 51 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 52 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

Figure 53 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidine uridine, 2'-C-methoxycarboxymethylidine uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 54 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine phosphoramidites.

Figure 55 is a diagrammatic representation of the synthesis of 2'-O-alkylthioalkyl nucleosides or non-nucleosides and their phosphoramidites. R is an alkyl as defined above. B is any naturally occuring or modified base bearing any N-protecting group suitable for standard oligonucleotide synthesis (Usman et al., supra; Scaringe et al., supra), and/or H (non-nucleotide), as described by the publications discussed above. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

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Figure 56 is a diagrammatic representation of a hammerhead ribozyme, targeted to site B (HH-B), containing 2'-O-methylthiomethyl substitutions.

Figure 57 shows RNA cleavage activity catalyzed by 2'-O-methylthiomethyl substituted ribozymes. A plot of percent cleaved as a function of time is shown. The reactions were carried out at 37°C in the presence of 40 nM ribozyme, 1 nM substrate and 10 mM MgCl₂. Control HH-B ribozyme contained the following modifications; 29 positions were modified with 2'-O-methyl, U4 and U7 positions were modified with 2'-amino groups, 5 positions contained 2'-OH groups. These modifications of the control ribozyme have previously been shown not to significantly effect the activity of the ribozyme (Usman et al., 1994 Nucleic Acids Symposium Series 31, 163).

Figure 58 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figure 59 is a diagrammatic representation of a hammerhead ribozyme targeted to site B (HH-B). Arrow indicates the cleavage site.

Figure 60 is a diagrammatic representation of HH-B ribozyme containing abasic substitutions (HH-Ba) at various positions. Ribozymes were synthesized as described in the application. "X" shows the positions of abasic substitutions. The abasic substitutions were either made individually or in certain combinations.

Figure 61 shows the *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. All RNA, refers to HHA ribozyme containing no abasic substitution. U4 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 4. U7 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 7.

Figure 62 shows in vitro RNA cleavage activity of HH-B and HH-Ba ribozymes. Abasic Stem II Loop, refers to HH-Ba ribozyme with four abasic (ribose) substitutions within the loop in stem II.

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Figure 63 shows in vitro RNA cleavage activity of HH-B and HH-Ba ribozymes. 3'-Inverted Deoxyribose, refers to HH-Ba ribozyme with an inverted deoxyribose (abasic) substitution at its 3' termini.

Figure 64 is a diagrammatic representation of a hammerhead ribozyme targeted to site A (HH-A). Target A is involved in the proliferation of mammalian smooth muscle cells. Arrow indicates the site of cleavage. Inactive version of HH-A contains 2 base-substitutions (G5U and A15.1U) that renders the ribozyme catalytically inactive.

Figure 65 is a diagrammatic representation of HH-A ribozyme with abasic substitution (HH-Aa) at position 4. X, shows the position of abasic substitution.

Figure 66 shows ribozyme-mediated inhibition of rat aortic smooth muscle cell (RASMC) proliferation. Both HH-A and HH-Aa ribozymes can inhibit the proliferation of RASMC in culture. Catalytically inactive HH-A ribozyme shows inhibition which is significantly lower than active HH-A and HH-Aa ribozymes.

Figure 67 is a schematic representation of a two pot deprotection protocol with ethylamine (EA).

Figure 68 shows a strategy used in synthesizing a hammerhead ribozyme from two halves. X and Y represent reactive moieties that can undergo a chemical reaction to form a covalent bond (represented by the solid curved line).

Figure 69 shows various non-limiting examples of reactive moieties that can be placed in the nascent loop region to form a covalent bond to provide a full-length ribozyme. CH2 can be any linking chain as described above including groups such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl and other functional groups or conjugates such as peptides, steroids, hoemones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization.

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Figure 70 shows non-limiting examples of covalent bonds that can be formed to provide the full length ribozyme. The morpholino group arises from reductive reaction of a dialdehyde, which arises from oxidative cleavage of a ribose at the 3'-end of one half ribozyme with an amine at the 5'-end of the half ribozyme. The amide bond is produced when an acid at the 3'-end of one half ribozyme is coupled to an amine at the 5'-end of the other half ribozyme.

Figure 71 shows non-limiting examples of three ribozymes that were synthesized from coupling reactions of two halves. All three were targeted to the site A of c-myb RNA (HH-A). HH-A1 was formed from the reaction of two thiols to provide the disulfide linked ribozyme. HH-A2 and HH-A3 were formed using the morpholino reaction. HH-A2 contains a five atom spacer linking the terminal amine to the 5'-end of the half ribozyme. HH-A3 contains a six carbon spacer linking the terminal amine to the 5'-end of the half ribozyme.

Figure 72 shows comparative cleavage activity of half ribozymes, containing five and six base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were corried out under ribozyme excess conditions.

Figure 73 shows comparative cleavage activity of half ribozymes, containing seven and eight base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 74 shows comparative cleavage assay of HH-A1, HH-A2 and HH-A3 (see Figure 72) formed from crosslinking reactions vs a full length ribozyme control. Assays were carried out under ribozyme excess conditions.

Figure 75. Schematic representation of RNA polymerse III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-

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acting sequence element that has not been fully characterized: EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figure 76 is a general formula for pol III RNA of this invention.

Figure 77 is a diagrammatic representation of a U6-S35 Chimera. The S35 motif and the site of insertion of a desired RNA are indicated. This chimeric RNA transcript is under the control of a U6 small nuclear RNA (snRNA) promoter.

Figure 78 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site I (HHI).

Figure 79 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site II (HHII).

Figure 80 shows RNA cleavage reaction catalyzed by a synthetic hammerhead ribozyme (HHI) and by an in vitro transcript of U6-S35-HHI hammerhead ribozyme.

Figure 81 shows stability of U6-S35-HHII RNA transcript in 293 mammalian cells as measured by actinomycin D assay.

Figure 82 is a diagrammatic representation of an adenovirus VA1 RNA.

Various domains within the RNA secondary structure are indicated.

Figure 83 A shows a secondary structure model of a VA1-S35 chimeric RNA containing the promoter elements A and B box. The site of insertion of a desired RNA and the S35 motif are indicated. The transcription unit also contains a stable stem (S35-like motif) in the central domain of the VA1 RNA which positions the desired RNA away from the main transcript as an independent domain. 83B shows a VA1-chimera which consists of the terminal 75 nt of a VA1 RNA followed by the HHI ribozyme.

Figure 84 shows a comparison of stability of VA1-chimeric RNA vs VA1-S35-chimeric RNA as measured by actinomycin D assay. VA1-chimera

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consists of terminal 75 nt of VA1 RNA followed by HHI ribozyme. VA1-S35-chimera structure and sequence is shown in Figure 83.

Ribozymes

Ribozymes in one aspect of this invention block to some extent stromelysin expression and can be used to treat disease or diagnose such disease. Ribozymes are delivered to cells in culture and to cells or tissues in animal models of osteoarthritis (Hembry et al., 1993 Am. J. Pathol. 143, 628). Ribozyme cleavage of stromelysin encoding mRNAs in these systems may prevent inflammatory cell function and alleviate disease symptoms.

Other ribozymes of this invention block to some extent B7-1, B7-2, B7-3 and/or CD40 production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture, to cells or tissues in animal models of transplantation, autoimmune diseases and/or allergies and to human cells or tissues *ex vivo* or *in vivo*. Ribozyme cleavage of B7-1, B7-2 and/or CD40 encoded mRNAs in these systems may alleviate disease symptoms.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al <u>supra</u>, Sullivan et al., <u>supra</u>, as well as by Draper et al., WO 95/13380 and Stinchcomb et al WO 95/23225. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized and delivered as described therein. While specific examples to mouse, rabbit and other animal RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

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The sequence of human and rabbit stromelysin mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables All, All, AV, AVI, AVIII and AIX (All sequences are 5' to 3' in the tables.). While rabbit and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, rabbit targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Similarly, the sequence of human and mouse B7-1, B7-2, B7-3 and/or CD40 mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVIII and BXIX (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

30 Referring to Figure 6, mRNA is screened for accessible cleavage sites by the method described generally in Draper WO 93/23569. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to

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generate a substrate for T7 RNA polymerase transcription from human or rabbit stromelysin cDNA clones. Labeled RNA transcripts are synthesized in vitro from the two templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead ribozyme sites are chosen as the most accessible.

10 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441; Wincott et al., 1995 Nucleic Acids Res. 23, 2677, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino. 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34 and Beigelman et al., 1995 J. Biol. Chem. 270, 25702). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb et al, supra) and are resuspended in water.

30 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables AV, AVII, AVIII and AIX and in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the

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binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables AV and AVII (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop AIV sequence of hairpin ribozymes listed in Tables AVI and AVII (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables AV, AVII, AVIII and AIX may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Stinchcomb et al., supra. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Stinchcomb et al., supra. Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings et al., WO 94/13688 and Beigelman et al., supra which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., <u>supra</u>, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some

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indications, ribozymes may be directly delivered ex vivo to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

In another preferred embodiment, the ribozyme is administered to the site of B7-1, B7-2, B7-3 and/or CD40 expression (APC) in an appropriate liposomal vesicle. APCs isolated from donor (for example) are treated with the ribozyme preparation (or other nucleic acid therapeutics) *ex vivo* and the treated cells are infused into recipient. Alternatively, cells, tissues or organs are directly treated with nucleic acids of the present invention prior to transplantation into a recipient.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. USA, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisziewicz et

al., 1993 <u>Proc. Natl. Acad. Sci. U. S. A.</u>, 90, 8000-4; Thompson *et al.*, <u>supra</u>). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adenoassociated vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves stromelysin RNA is inserted into a plasmid DNA vector or an adenovirus DNA virus or adeno-associated virus (AAV) vector. Both viral vectors have been used to transfer genes to the lung and both vectors lead to transient gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, e.g., through incubation or inhalation in vivo or by direct application to cells or tissues ex vivo.

Specifically useful modifications, optimization and synthetic methods will now be described.

20 Base Modifications

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The following discussion of relevant art is dependent on the diagram shown in Figure 1, in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

Odai et al., FEBS 1990, 267:150, state that substitution of guanosine (G) at position 5 of a hammerhead ribozyme for inosine greatly reduces catalytic activity, suggesting "the importance of the 2-amino group of this guanosine for catalytic activity."

Fu and McLaughlin, Proc. Natl. Acad. Sci. (USA) 1992, 89:3985, state that deletion of the 2-amino group of the guanosine at position 5 of a hammerhead ribozyme, or deletion of either of the 2'-hydroxyl groups at

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position 5 or 8, resulted in ribozymes having a decrease in cleavage efficiency.

Fu and McLaughlin, *Biochemistry* 1992, *31*:10941, state that substitution of 7-deazaadenosine for adenosine residues in a hammerhead ribozyme can cause reduction in cleavage efficiency. They state that the "results suggest that the N⁷-nitrogen of the adenosine (A) at position 6 in the hammerhead ribozyme/substrate complex is critical for efficient cleavage activity." They go on to indicate that there are five critical functional groups located within the tetrameric sequence GAUG in the hammerhead ribozyme.

Slim and Gait, 1992, BBRC 183, 605, state that the substitution of guanosine at position 12, in the core of a hammerhead ribozyme, with inosine inactivates the ribozyme.

Tuschl et al., 1993 Biochemistry 32, 11658, state that substitution of guanosine residues at positions 5, 8 and 12, in the catalytic core of a hammerhead, with inosine, 2-aminopurine, xanthosine, isoguanosine or deoxyguanosine cause significant reduction in the catalytic efficiency of a hammerhead ribozyme.

Fu et al., 1993 Biochemistry 32, 10629, state that deletion of guanine N^7 , guanine N^2 or the adenine N^6 -nitrogen within the core of a hammerhead ribozyme causes significant reduction in the catalytic efficiency of a hammerhead ribozyme.

Grasby et al., 1993 Nucleic Acids Res. 21, 4444, state that substitution of guanosine at positions 5, 8 and 12 positions within the core of a hammerhead ribozyme with O⁶-methylguanosine results in an approximately 75-fold reduction in k_{Cat}.

Seela et al., 1993 Helvetica Chimica Acta 76, 1809, state that substitution of adenine at positions 13, 14 and 15, within the core of a hammerhead ribozyme, with 7-deazaadenosine does not significantly decrease the catalytic efficiency of a hammerhead ribozyme.

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Adams et al., 1994 Tetrahedron Letters 35, 765, state that substitution of uracil at position 17 within the hammerhead ribozyme-substrate complex with 4-thiouridine results in a reduction in the catalytic efficiency of the ribozyme by 50 percent.

Ng et al., 1994 Biochemistry 33, 12119, state that substitution of adenine at positions 6, 9 and 13 within the catalytic core of a hammerhead ribozyme with isoguanosine, significantly decreases the catalytic activity of the ribozyme.

Jennings et al., U.S. Patent 5,298,612, indicate that nucleotides within a "minizyme" can be modified. They state-

"Nucleotides comprise a base, sugar and a monophosphate group. Accordingly, nucleotide derivatives or modifications may be made at the level of the base, sugar or monophosphate groupings..... Bases may be substituted with various groups, such as halogen, hydroxy, amine, alkyl, azido, nitro, phenyl and the like."

WO93/23569, WO95/06731, WO95/04818, and WO95/133178 describe various modifications that can be introduced into ribozyme structures.

This invention relates to production of enzymatic RNA molecules or ribozymes having enhanced or reduced binding affinity and enhanced enzymatic activity for their target nucleic acid substrate by inclusion of one or more modified nucleotides in the substrate binding portion of a ribozyme such as a hammerhead, hairpin, VS ribozyme or hepatitis delta virus derived ribozyme. Applicant has recognized that only small changes in the extent of base-pairing or hydrogen bonding between the ribozyme and substrate can have significant effect on the enzymatic activity of the ribozyme on that substrate. Thus, applicant has recognized that a subtle alteration in the extent of hydrogen bonding along a substrate binding arm of a ribozyme can be used to improve the ribozyme activity compared to an unaltered ribozyme containing no such altered nucleotide. Thus, for example, a guanosine base may be replaced with an inosine to produce a weaker interaction between a ribozyme and its substrate, or a uracil may be replaced with a bromouracil (BrU) to increase the hydrogen bonding interaction with an adenosine. Other

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examples of alterations of the four standard ribonucleotide bases are shown in Figures 22a-d with weaker or stronger hydrogen bonding abilities shown in each figure.

In addition, applicant has determined that base modification within some catalytic core nucleotides maintains or enhances enzymatic activity compared to an unmodified molecule. Such nucleotides are noted in Figure 23. Specifically, referring to Figure 23, the preferred sequence of a hammerhead ribozyme in a 5' to 3' direction of the catalytic core is CUG ANG A G•C GAA A, wherein N can be any base or may lack a base (abasic); G•C is a base-pair. The nature of the base-paired stem II (Figures 1, 2 and 23) and the recognition arms of stems I and III are variable. In this invention, the use of base-modified nucleotides in those regions that maintain or enhance the catalytic activity and/or the nuclease resistance of the hammerhead ribozyme are described. (Bases which can be modified include those shown in capital letters).

Examples of base-substitutions useful in this invention are shown in Figure 22, 24-30, 39-43, 45-46. In preferred embodiments cytidine residues are substituted with 5-alkylcytidines (e.g., 5-methylcytidine, Figure 24, R=CH3, 9), and uridine residues with 5-alkyluridines (e.g., ribothymidine (Figure 24, R=CH3, 4) or 5-halouridine (e.g., 5-bromouridine, Figure 24, X=Br, 13) or 6-azapyrimidines (Figure 24, 17) or 6-alkyluridine (Figure 30). Guanosine or adenosine residues may be replaced by diaminopurine residues (Figure 24, 22) in either the core or stems. In those bases where none of the functional groups are important in the complexing of magnesium or other functions of a ribozyme, they are optionally replaced with a purine ribonucleoside (Figure 24, 23), which significantly reduces the complexity of chemical synthesis of ribozymes, as no base-protecting group is required during chemical incorporation of the purine nucleus. Furthermore, as discussed above, base-modified nucleotides may be used to enhance the specificity or strength of binding of the recognition arms with similar modifications. Base-modified nucleotides, in general, may also be used to enhance the nuclease resistance of the catalytic nucleic acids in which they are incorporated. modifications within the hammerhead ribozyme motif are meant to be nonlimiting example. Those skilled in the art will recognize that other ribozyme

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motifs with similar modifications can be readily synthesized and are within the scope of this invention.

Substitutions of sugar moieties as described in the art cited above, may also be made to enhance catalytic activity and/or nuclease stability.

The invention provides ribozymes having increased enzymatic activity in vitro and in vivo as can be measured by standard kinetic assays. Thus, the kinetic features of the ribozyme are enhanced by selection of appropriate modified bases in the substrate binding arms. Applicant recognizes that while strong binding to a substrate by a ribozyme enhances specificity, it may also prevent separation of the ribozyme from the cleaved substrate. Thus, applicant provides means by which optimization of the base pairing can be achieved. Specifically, the invention features ribozymes with modified bases with enzymatic activity at least 1.5 fold (preferably 2 or 3 fold) or greater than the unmodified corresponding ribozyme. The invention also features a method for optimizing the kinetic activity of a ribozyme by introduction of modified bases into a ribozyme and screening for those with higher enzymatic activity. Such selection may be in vitro or in vivo. By enhanced activity is meant to include activity measured in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of 20 these properties in increased or not significantly (less that 10 fold) decreased in vivo compared to an all RNA ribozyme.

By "enzymatic portion" is meant that part of the ribozyme essential for cleavage of an RNA substrate.

By "substrate binding arm" is meant that portion of a ribozyme which is complementary to (i.e., able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme 30 and target RNA together through complementary base-pairing interactions; e.g., ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

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By "unmodified nucleotide base" is meant one of the bases adenine, cytosine, guanosine, uracil joined to the 1' carbon of B-D-ribo-furanose. The sugar also has a phosphate bound to the 5' carbon. These nucleotides are bound by a phosphodiester between the 3' carbon of one nucleotide and the 5' carbon of the next nucleotide to form RNA.

By "modified nucleotide base" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base which has an effect on the ability of that base to hydrogen bond with its normal complementary base, either by increasing the strength of the hydrogen bonding or by decreasing it (e.g., as exemplified above for inosine and bromouracil). Other examples of modified bases include those shown in Figures 22a-d and other modifications well known in the art, including heterocyclic derivatives and the like.

In preferred embodiments the modified ribozyme is a hammerhead, hairpin VS ribozyme or hepatitis delta virus derived ribozyme, and the hammerhead ribozyme includes between 32 and 40 nucleotide bases. The selection of modified bases is most preferably chosen to enhance the enzymatic activity (as observed in standard kinetic assays designed to measure the kinetics of cleavage) of the selected ribozyme, *i.e.*, to enhance the rate or extent of cleavage of a substrate by the ribozyme, compared to a ribozyme having an identical nucleotide base sequence without any modified base.

By "kinetic assays" or "kinetics of cleavage" is meant an experiment in which the rate of cleavage of target RNA is determined. Often a series of assays are performed in which the concentrations of either ribozyme or substrate are varied from one assay to the next in order to determine the influence of that parameter on the rate of cleavage.

By "rate of cleavage" is meant a measure of the amount of target RNA cleaved as a function of time.

Enzymatic nucleic acid having a hammerhead configuration and modified bases which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than

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unmodified nucleic acid. By "modified bases" in this aspect is meant those shown in Figure 22 A-D, and 24, 30, and 42B or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in vivo the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in vivo even if active over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5 μ mol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table CII outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μL of 0.1 M = 16.3 μmol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μ L of 0.25 M = 59.5 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer: detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of

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EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA•HF/NMP solution (250 μL of a solution of 1.5mL N-methylpyrrolidinone, 750 μL TEA and 1.0 mL TEA•3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

Inactive hammerhead ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from (Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, ::252)).

The average stepwise coupling yields were >98% (Wincott et al., 1995 Nucleic Acids Res. 23, 2677-2684).

Hairpin ribozymes are synthesized either as one part or in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840).

Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb *et al.*, International PCT Publication No. WO 95/23225, and are resuspended in water.

Various modifications to ribozyme structure can be made to enhance the utility of ribozymes. Such modifications will enhance shelf-life, half-life in vitro, stability, and ease of introduction of such ribozymes to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

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Examples of such ribozymes are provided in Usman et al., WO 95/13378 and below.

2'deoxy-2'-nucleotides

Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and 5 Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Stinchcomb et al., supra, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings et al., WO 94/13688 and Beigelman et al., supra which describe various chemical 10 modifications that can be made to the sugar moieties of enzymatic RNA molecules. Usman et al. also describe various required ribonucleotides in a ribozyme, and methods by which such nucleotides can be defined. De Mesmaeker et al. Syn. Lett. 1993, 677-680 (not admitted to be prior art to the present invention) describes the synthesis of certain 2'-C-alkyl uridine and 15 thymidine derivatives. They conclude that "...their use in an antisense approach seems to be very limited."

This invention relates to the use of 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic acid molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker et al. applicant has found that such

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nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures 48-54), and to methods for their synthesis.

Thus, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiet and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 48, where each R group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may

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be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =0, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =0, =S, NO₂ or N(CH₃)₂, amino or SH. The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are ...alogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above. Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an

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enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

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The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonulceotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 47 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 47, and the binding arms correspond to the ses from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman et al. supra.

2'-0-alkylthioalkyl and 2'-C-alkylthioalkyl containing nucleic acids

Medina et al., 1988 *Tetrahedron Letters* 29, 3773, describe a method to convert alcohols to methylthiomethyl ethers.

Matteucci et al., 1990 *Tetrahedron Letters*, 31, 2385, report the synthesis of 3'-5'-methylene bond via a methylthiomethyl precursor.

Veeneman et al., 1990 Recl. Trav. Chim. Pays-Bas 109, 449, report the synthesis of 3'-O-methylthiomethyl deoxynucleoside during the synthesis of a dimer containing 3'-5'-methylene bond.

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Jones et al., 1993 J. Org. Chem. 58, 2983, report the use of 3'-O-methylthiomethyl deoxynucleoside to synthesize a dimer containing a 3'-thioformacetal internucleoside linkages. The paper also describes a method to synthesize phosphoramidites for DNA synthesis.

Zavgorodny et al., 1991 Tetrahedron Letters 32, 7593, describe a method to synthesize a nucleoside containing methylthiomethyl modification.

This invention relates to the incorporation of 2'-O-alkyllthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides into nucleic acids, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides.

As the term is used in this application, 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotides components replacing one or more bases or regions including, but not limited to, those bases in double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides which may be present in enzymatic nucleic acid or in antisense oligonucleotides or 2-5A antisense chimera. Such nucleotides or non-nucleotides are useful since they enhance the activity of the antisense or enzymatic molecule. The invention also relates to novel intermediates useful in the synthesis of such nucleotides or non-nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, the invention features 2'-O-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleoside or non-nucleosides having at the 2'-position on the sugar molecule a 2'-O-alkylthioalkyl moiety. In a related aspect, the

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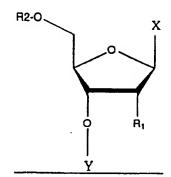
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invention also features 2'-O-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes those nucleotides or non-nucleotides having 2' substitutions as noted above useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

The term non-nucleotide refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2' or 3' H or OH as described in the art. See Eckstein et al. and Usman et al., supra.

The term nucleotide refers to the regular nucleotides (A, U, G, T and C) and modified nucleotides such as 6-methyl U, inosine, 5-methyl C and others. Specifically, the term "nucleotide" is used as recognized in the art to include natural bases, and modifie — ses well known in the art. Such bases are generally located at the 1' position of a sugar moiety. The term "non-nucleotide" as used herein to encompass sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position. Such molecules generally include those having the general formula:



wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents H, DMT or a phosphorus-containing group (Figure 55).

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Phosphorus-containing group is generally a phosphate, thiophosphate, H-phosphonate, methylphosphonate, phosphoramidite or other modified group known in the art.

In a another aspect, the invention features 2'-C-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleotide or a non-nucleotide residue having at the 2'-position on the sugar molecule a 2'-C-alkylthioalkyl moiety. In a related aspect, the invention also features 2'-C-alkylthioalkyl nucleotides or nonnucleotides. That is, the invention preferably includes all those 2' modified nucleotides or non-nucleotides useful for making enzymatic nucleic acids or antisense molecules as described above that are not described by the art discussed above.

Specifically, an "alkyl" group is as defined above, except that the term includes 2'-O-alkyl moeities.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-Calkylthioalkyl nucleotides or non-nucleotides; e.g. enzymatic nucleic acids having a 2'-O-methylthiomethyl ai. /or 2'-C-alkylthioalkyl nucleotides or nonnucleotides; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, 20 by forming the enzymatic molecule with at least one nucleotide or a nonnucleotide moiety having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-Calkylthioalkyl group.

In other related aspects, the invention features 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl derivatives of this invention provide enhanced activity and stability to the oligonulceotides containing them.

In yet another preferred embodiment, the invention features 30 oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-Calkylthioalkyl abasic (non-nucleotide) moeities. For example, enzymatic

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nucleic acids having a 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic moeity; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl group.

In related embodiments, the invention features enzymatic nucleic acids containing one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substitutions either in the enzymatic portion, substrate binding portion or both, as long as the catalytic activity of the ribozyme is not significantly decreased.

In yet another preferred embodiment, the invention features the use of 2'-O-alkylthioalkyl moieties as protecting groups for 2'-hydroxyl positions of ribofuranose during nucleic acid synthesis.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particulary useful for enzymatic RNA molecules. Thus, below is provided examples of such molecules. Those in the art will recognith that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG [base paired with] CGAAA. In this invention, the use of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substituted nucleotides or non-nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides or non-nucleotides discussed above are possible. Usman et al., supra and Sproat et al., supra as well as other publications indicate those bases that can be substituted in noted ribozyme motifs. Those in the art can thus determine those bases that may be substituted as described herein with beneficial retainment of enzymatic activity and stability.

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Non-nucleotides

Usman, et al., WO 93/15187 in discussing modified structures in ribozymes states:

It should be understood that the linkages between the building units of the polymeric chain may be linkages capable of bridging the units together for either in vitro or in vivo. For example the linkage may be a phosphorous containing linkage, e.g., phosphodiester or phosphothioate, or may be a nitrogen containing linkage, e.g., amide. It should further be understood that the chimeric polymer may contain non-nucleotide spacer molecules along with its other nucleotide or analogue units. Examples of spacer molecules which may be used are described in Nielsen et al. Science, 254:1497-1500 (1991).

Jennings et al., WO 94/13688 while discussing hammerhead ribozymes lacking the usual stem II base-paired region state:

One or more ribonucleotides and/or deoxyribonucleotides of the group (X)m. [stem II] may be replaced, for example, with a linker selected optionally from substituted polyphosphodiester (such as poly(1-phospho-3propanol)), optionally substituted alkyl, optionally substituted polyamide, optionally substituted glycol, and the like. Optional substituents are well known in the art, and include alkoxy (such as methoxy, ethoxy and propoxy), straight or branch chain lower alkyl such as C1 - C5 alkyl), amine, aminoalkyl (such as amino C1 - C5 alkyl), halogen (such as F, C1 and Br) and the like. The nature of optional substituents is not of importance, as long as the resultant endonuclease is capable of substrate cleavage.

Additionally, suitable linkers may comprise polycyclic molecules, such as those containing phenyl or cyclohexyl rings. The linker (L) may be a polyether such as polyphosphopropanediol, polyethyleneglycol, a bifunctional polycyclic molecule such as a bifunctional pentalene, indene, naphthalene, azulene, heptalene, biphenylene, asymindacene, sym-indacene, acenaphthylene, fluorene, phenalene, phenanthrene, anthracene, fluoranthene, acephenathrylene, aceanthrylene,

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triphenylene, pyrene, chrysene, naphthacene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, indolizine, isoindole, 3-H-indole, indole, 1-H-indazole, 4-H-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4-\alphaH-carbzole, carbazole, B-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenolthiazine, phenoxazine, which polycyclic compound may be substituted or modified, or a combination of the polyethers and the polycyclic molecules.

The polycyclic molecule may be substituted of polysubstituted with C1 -C5 alkyl, alkenyl, hydroxyalkyl, halogen of haloalkyl group or with O-A or CH2-O-A wherein A is H or has the formula CONR'R" wherein R' and R" are the same or different and are hydrogen or a substituted or unsubstituted C1 - C6 alkyl, aryl, cycloalkyl, or heterocyclic group; or A has the formula -M-NR'R" wherein R' and R" are the same or different and are hydrogen, or a C1-C5 alkyl, alkenyl, hydroxyalkyl, or haloalkyl group wherein the halo atom is fluorine, chlorine, bromine, or iodine at m; and -M-is an organic moiety having 1 to 10 c ion atoms and is a branched or straight chain alkyl, aryl, or cycloalkyl group.

In one embodiment, the linker is tetraphosphopropanediol. In the case of polycyclic molecules there will be preferably 18 or more atoms bridging the nucleic acids. More preferably their will be from 30 to 50 atoms bridging, see for Example 5. In another embodiment the linker is a bifunctional carbazole or bifunctional carbazole linked to one or more polyphosphoropropanediol.

Such compounds may also comprise suitable functional groups to allow coupling through reactive groups on nucleotides."

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (e.g., RNA or DNA) stems (duplex stems) or more preferably, in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex

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stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme. In particular abasic nucleotides (i.e., moieties lacking a nucleotide base, but having the sugar and phosphate portions) can be used to provide stability within a core of a ribozyme, e.g., at U4 or N7 of a hammerhead structure shown in Figure 1.

Thus, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

Examples of such non-nucleotide mimetics are shown in Figure 58 and their incorporation into hammerhead ribozymes is shown in Figure 60. These 15 non-nucleotide linkers may be either polyether, polyamine, polyamide, or polyhydrocarbon compounds. Specific examples include titose described by Seela and Kaiser, Nucleic Acids Res. 1990, 18:6353 and Nucleic Acids Res. 1987, 15:3113; Cload and Schepartz, J. Am. Chem. Soc. 1991, 113:6324; Richardson and Schepartz, J. Am. Chem. Soc. 1991, 113:5109; Ma et al., 20 Nucleic Acids Res. 1993, 21:2585 and Biochemistry 1993, 32:1751; Durand et al., Nucleic Acids Res. 1990, 18:6353; McCurdy et al., Nucleosides & Nucleotides 1991, 10:287; Jäschke et al., Tetrahedron Lett. 1993, 34:301; Ono et al., Biochemistry 1991, 30:9914; Amold et al., International Publication No. WO 89/02439 entitled "Non-nucleotide Linking Reagents for Nucleotide Probes"; and Ferentz and Verdine, J. Am. Chem. Soc. 1991, 113:4000, all hereby incorporated by reference herein.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule,

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linked to the non-nucleotide moiety. The necessary ribonucleotide components are known in the art, <u>see</u>, e.g., Usman, <u>supra</u> and Usman et al., <u>Nucl. Acid. Symp. Genes</u> 31:163, 1994.

As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, e.g., but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3' or 5' non-nucleotide, useful as a capping group to prevent exonuclease digestion.

Non-nucleotide mimetics useful in this invention are generally described above and in Usman et al. WO 95/06731. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecu! hy standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, e.g., by synthesis of the molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule synthesis and to provide enhanced stability of the molecule compared to RNA or even DNA.

Synthesis

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity. Such syntheses are generally detailed in Stinchcomb t al., WO 95/23225.

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This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity.

Generally, RNA is synthesized and purified by methodologies based on: tetrazole to activate the RNA amidite, NH₄OH to remove the exocyclic amino protecting groups, tetra-n-butylammonium fluoride (TBAF) to remove the 2'-OH alkylsilyl protecting groups, and gel purification and analysis of the deprotected RNA. In particular this applies to, but is not limited to, a certain class of RNA molecules, ribozymes. These may be formed either chemically or using enzymatic methods. Examples of the chemical synthesis, deprotection, purification and analysis procedures are provided by Usman et al., 1987 J. American Chem. Soc., 109, 7845, Scaringe et al. Nucleic Acids Res. 1990, 18, 5433-5341, Perreault et al. Biochemistry 1991, 30 4020-4025, and Slim and Gait Nucleic Acids Res. 1991, 19, 1183-1188. Odai et al. FEBS Lett. 1990, 267, 150-152 describes a reverse phase chromatographic purification of RNA fragments used to form a ribozyme. All the above noted references are all hereby incorporated by reference herein.

The aforementioned chemical synthesis, deprotection, purification and analysis procedures are time consuming (10-15 m coupling times) and may also be affected by inefficient activation of the RNA amidites by tetrazole, time consuming (6-24 h) and incomplete deprotection of the exocyclic amino protecting groups by NH₄OH, time consuming (6-24 h), incomplete and difficult to desalt TBAF-catalyzed removal of the alkylsilyl protecting groups, time consuming and low capacity purification of the RNA by gel electrophoresis, and low resolution analysis of the RNA by gel electrophoresis.

Imazawa and Eckstein, 1979 J. Org. Chem., 12. 2039, describe the synthesis of 2'-amino-2'-deoxyribofuranosyl purines. They state that—

"To protect the 2'-amino function, we selected the trifluoroacetyl group which can easily be removed."

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Chemical linkage

Jennings et al., US Patent No. 5,298,612 describe the use of non-nucleotides to assemble a hammerhead ribozyme lacking a stem II portion.

Draper et al., WO 93/23569 (PCT/US93/04020) describes synthesis of ribozymes in two parts in order to aid in the synthetic process (see, e.g., p. 40).

Usman et al., WO 95/06731, describe enzymatic nucleic acid molecules having non-nucleotides within their structure. Such non-nucleotides can be used in place of nucleotides to allow formation of an enzymatic nucleic acid.

This invention relates to improved methods for synthesis of enzymatic nucleic acids and, in particular, hammerhead and hairpin motif ribozymes. This invention is advantageous over iterative chemical synthesis of ribozymes since the yield of the final ribozyme can be significantly increased. Rather than synthesizing, for example, a 37mer hammerhead ribozyme, two partial ribozyme portions, e.g., a 20mer and a 17mer, can be synthesized in significantly higher yield, and the two reacted together to form the desired enzymatic nucleic acid.

Referring to Fig. 68, the strategy involved is shown for a hammerhead ribozyme where each n or n' is independently any desired nucleotide or nonnucleotide, each filled-in circle represents pairing between bases or other entities, and the solid line represents a covalent bond. Within the structure each n and n' may be a ribonucleotide, a 2'-methoxy-substituted nucleotide, or any other type of nucleotide which does not significantly affect the desired enzymatic activity of the final product (see Usman et al., supra). In the particular embodiment shown, which is not limiting in this invention, five ribonucleotides are provided at rG5, rA6, rG8, rG12, and rA15.1. U4 and U7 may be abasic (i.e., lacking the uridine moiety) or may be ribonucleotides, 2'methoxy substituted nucleotides, or other such nucleotides. a9, a13, and a14 are preferably 2'-methoxy or may have other substituents. The synthesis of this hammerhead ribozyme is performed by synthesizing a 3' and a 5' portion as shown in a lower part of Fig. 68. Each 5' and 3' portion has a chemically reactive group X and Y, respectively. Non-limiting examples of such chemically reactive groups are provided in Fig. 69. These groups undergo

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chemical reactions to provide the bonds shown in Fig. 69. Thus, the X and Y can be used, in various combinations, in this invention to form a chemical linkage between two ribozyme portions.

Thus, the invention features a method for synthesis of an enzymatically active nucleic acid (as defined by Draper, <u>supra</u>) by providing a 3' and a 5' portion of that nucleic acid, each having independently chemically reactive groups at the 5' and 3' positions, respectively. The reaction is performed under conditions in which a covalent bond is formed between the 3' and 5' portions by those chemically reactive groups. The bond formed can be, but is not limited to, either a disulfide, morpholino, amide, ether, thioether, amine, a double bond, a sulfonamide, carbonate, hydrazone or ester bond. The bond is not the natural bond formed between a 5' phosphate group and a 3' hydroxyl group which is made during normal synthesis of an oligonucleotide. In other embodiments, more than two portions can be linked together using pairs of X and Y groups which allow proper formation of the ribozyme (see Figure 69).

By "chemically reactive group" is simply meant a group which can react with another group to form the desired bonds. These bonds may be formed under any conditions which will not significantly affect the structure of the resulting enzymatic nucleic acid. Those in the art will recognize that suitable protecting groups can be provided on the ribozyme portions.

In preferred embodiments the nucleic acid has a hammerhead motif and the 3' and 5' portions each have chemically reactive groups in or immediately adjacent to the stem II region (see Fig. 1). The stem II region is evident in Fig. 1 between the bases termed a9 and rG12. The C and G within this stem defines the end of the stem II region. Thus, any of the n or n' moieties within the stem II region can be provided with a chemically reactive group. As is evident from this structure, the chemically reactive groups need not be provided in the solid line portion but can be provided at any of the n or n'. In this way the length of each of the 5' and 3' portions can vary by several bases (Figure 70).

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In other preferred embodiments, the chemically reactive group can be, but is not limited to, (CH₂)_nSH; (CH₂)_nNHR; (CH₂)_nX; ribose; COOH; (CH₂)_nPPh₃; (CH₂)_nSO₂Cl; (CH₂)_nCOR; (CH₂)_nRNH or (CH₂)_nOH, where, CH₂ can be replaced by another group which forms a linking chain (which does not interfere with the terminal chemically reactive group) containing various atoms including, but not limited to CH₂, such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others, generally at most 20 such atoms are provided in the linking chain, most preferably only 5 - 10 atoms, and even more preferably only 3- 5 atoms; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl (as described above) and other functional groups or conjugates such as peptides, steroids, hoemones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization. X is halogen, and Ph represents a phenyl ring.

In yet other preferred embodiments, the conditions include provision of NaIO₄ in contact with the ribose, and subsequent provision of a reducing group such as NaBH₄ or NaCNBH₃; or the conditions include provision of a coupling reagent.

In a second related aspect, the invention features a mixture of the 5' and 3' portions of the enzymatically active nucleic acids having the 3' and 5' chemically reactive groups noted above.

Those in the art will recognize that while examples are provided of half ribozymes it is possible to provide ribozymes in 3 or more portions. For example, the hairpin ribozyme may be synthesized by inclusion of chemically reactive groups in helix IV and in other helices which are not critical to the enzymatic activity of the nucleic acid.

Pol III-based vectors

This invention relates to RNA polymerase III-based methods and systems for expression of therapeutic RNAs in cells *in vivo* or *in vitro*.

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The RNA polymerase III (pol III) promoter is one found in DNA encoding 5S, U6, adenovirus VA1, Vault, telomerase RNA, tRNA genes, etc., and is transcribed by RNA polymerase III (for a review see Geiduschek and Tocchini-Valentini, 1988 Annu. Rev. Biochem. 57, 873-914; Willis, 1993 Eur. J. Biochem. 212, 1-11). There are three major types of pol III promoters: types 1, 2 and 3 (Geiduschek and Tocchini-Valentini, 1988 supra; Willis, 1993 supra) (see Figure 1). Type 1 pol III promoter consists of three cis-acting sequence elements downstream of the transcriptional start site a) 5'sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). 5S ribosomal RNA genes are transcribed using the type 1 pol III promoter (Specht et al., 1991 Nucleic Acids Res. 19, 2189-2191.

The type 2 pol III promoter is characterized by the presence of two cisacting sequence elements downstream of the transcription start site. All Transfer RNA (tRNA), adenovirus VA RNA and Vault RNA (Kikhoefer et al., 1993, *J. Biol. Chem.* 268, 7868-7873) genes are transcribed using this promoter (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*). The sequence composition and orientation of the two cis-acting sequence elements- A box (5' sequence element) and B box (3' sequence element) are essential for optimal transcription by RNA polymerase III.

The type 3 pol III promoter contains all of the cis-acting promoter elements upstream of the transcription start site. Upstream sequence elements include a traditional TATA box (Mattaj et al., 1988 Cell 55, 435-442), proximal sequence element (PSE) and a distal sequence element (DSE; Gupta and Reddy, 1991 Nucleic Acids Res. 19, 2073-2075). Examples of genes under the control of the type 3 pol III promoter are U6 small nuclear RNA (U6 snRNA) and Telomerase RNA genes.

In addition to the three predominant types of pol III promoters described above, several other pol III promoter elements have been reported (Willis, 1993 supra) (see Figure 76). Epstein-Barr-virus-encoded RNAs (EBER), Xenopus seleno-cysteine tRNA and human 7SL RNA are examples of genes that are under the control of pol III promoters distinct from the aforementioned types of promoters. EBER genes contain a functional A and B box (similar to type 2 pol III promoter). In addition they also require an EBER-specific TATA

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box and binding sites for ATF transcription factors (Howe and Shu, 1989 *Cell* 57,825-834). The seleno-cysteine tRNA gene contains a TATA box, PSE and DSE (similar to type 3 pol III promoter). Unlike most tRNA genes, the seleno-cysteine tRNA gene lacks a functional A box sequence element. It does require a functional B box (Lee et al., 1989 *J. Biol. Chem.* 264, 9696-9702). The human 7SL RNA gene contains an unique sequence element downstream of the transcriptional start site. Additionally, upstream of the transcriptional start site, the 7SL gene contains binding sites for ATF class of transcription factors and a DSE (Bredow et al., 1989 *Gene* 86, 217-225).

Gilboa WO 89/11539 and Gilboa and Sullenger WO 90/13641 describe transformation of eucaryotic cells with DNA under the control of a pol III promoter. They state:

In an attempt to improve antisense RNA synthesis using stable gene transfer protocols, the use of pol III promoters to drive the expression of antisense RNA can be considered. The underlying rationale for the use of pol III promoters is that they can generate substantially higher levels of RNA transcripts in cells as compared to pol II promoters. For example, it is estimated that in a eucaryotic cell there are about 6 x 107 t-RNA molecules and 7 x 10^5 mRNA molecules, i.e., about 100 fold more pol III transcripts of this class than total pol II transcripts. Since there are about 100 active t-RNA genes per cell, each t-RNA gene will generate on the average RNA transcripts equal in number to total pol II transcripts. Since an abundant pol II gene transcript represents about 1% of total mRNA while an average pol II transcript represents about 0.01% of total mRNA, a t-RNA (pol III) based transcriptional unit may be able to generate 100 fold to 10,000 fold more RNA than a pol II based transcriptional unit. Several reports have described the use of pol III promoters to express RNA in eucaryotic cells. Lewis and Manley and Sisodia have fused the Adenovirus VA-1 promoter to various DNA sequences (the herpes TK gene, globin and tubulin) and used transfection protocols to transfer the resulting DNA constructs into cultured cells which resulted in transient synthesis of RNA in the transduced cell. De la Pena and Zasloff have expressed a t-RNA-Herpes TK fusion DNA construct upon microinjection into frog oocytes. Jennings and Molloy have constructed an antisense RNA template by fusing the VA-1 gene promofer to a DNA fragment derived from SV40 based vector which also resulted in transient expression of antisense RNA and limited inhibition of the target gene*. [Citations omitted.]

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The authors describe a fusion product of a chimeric tRNA and an RNA product (see Fig. 1C of WO 90/13641). In particular they describe a human tRNA meti derivative 3-5. 3-5 was derived from a cloned human tRNA gene by deleting 19 nucleotides from the 3' end of the gene. The authors indicate that the truncated gene can be transcribed if a termination signal is provided, but that no processing of the 3' end of the RNA transcript takes place.

Adeniyi-Jones et al.,1984 *Nucleic Acids Res.* 12, 1101-1115, describe certain constructions which "may serve as the basis for utilizing the tRNA gene as a 'portable promoter' in engineered genetic constructions." The authors describe the production of a so-called $\Delta 3'$ -5 in which 11 nucleotides of the 3'-end of the mature tRNAi^{met} sequence are replaced by a plasmid sequence, and are not processed to generate a mature tRNA. The authors state:

"the properties of the tRNAimet 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human tRNAimet gene. By fusion of the DNA sequence to a tRNA; met deletion mutant such as $\Delta 3'$ -4, a long read-through transcript would be generated in vivo (dependent, of course, on the absence of effective RNA polymerase III termination sequences). Fusion of the DNA sequence to a tRNA; met deletion mutant such as A3'-5 would lead to the generation of a co-transcript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by properties of mRNA species bearing tRNA sequences as 5' leaders in prokaryotes. Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu and may use the tRNA leaders as a means of stabilizing the transcript from degradation in vivo. The potential use of the tRNA; met gene as a *promoter leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted tRNA; met sequences contained on plasmids A 3'-4 and Δ 3'-5 in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both X. laevis oocytes and somatic cells". [References omitted].

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Sullenger et al., 1990 *Cell* 63, 601-619, describe over-expression of *TAR*-containing sequences using a chimeric tRNA_imet_*TAR* transcription unit in a double copy (DC) murine retroviral vector.

Sullenger et al., 1990 *Molecular and Cellular Bio.* 10, 6512, describe expression of chimeric tRNA driven antisense transcripts. It indicates:

"successful use of a tRNA-driven antisense RNA transcription system was dependent on the use of a particular type of retroviral vector, the double-copy (DC) vector, in which the chimeric tRNA gene was inserted in the viral LTR. The use of an RNA pol III-based transcription system to stably express high levels of foreign RNA sequences in cells may have other important applications. Foremost, it may significantly improve the ability to inhibit endogenous genes in eucaryotic cells for the study of gene expression and function, whether antisense RNA, ribozymes, or competitors of sequence-specific binding factors are used. tRNA-driven transcription systems may be particularly useful for introducing "mutations" into the germ line, i.e., for generating transgenic animals or transgenic plants. Since tRNA genes are ubiquitously expressed in all cell types, the chimeric tRNA genes may be properly expressed in all tissues of the animal, in contrast to the more idiosyncratic behavior of RNA pol II-based transcription units. However, homologous recombination represents a more elegant although, at present, very cumbersome approach for introducing mutations into the germ line. In either case, the ability to generate transgenic animals or plants carrying defined mutations will be an extremely valuable experimental tool for studying gene function in a developmental context and for generating animal models for human genetic disorders. In addition, tRNA-driven gene inhibition strategies may also be useful in creating pathogenresistant livestock and plants. [References omitted.]

Cotten and Birnstiel, 1989 EMBO Jml. 8, 3861, describe the use of tRNA genes to increase intracellular levels of ribozymes. The authors indicate that the ribozyme coding sequences were placed between the A and the B box internal promoter sequences of the Xenopus tRNA^{met} gene. They also indicate that the targeted hammerhead ribozymes were active in vivo.

Yu et al., 1993 *Proc. Natl. Acad. Sci.* USA 90, 5340, describe the use of a VAI promoter to express a hairpin ribozyme. The resulting transcript consisted

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of the first 104 nucleotides of the VAI RNA, followed by the ribozyme sequence and the terminator sequence.

Lieber and Strauss, 1995 Mol. Cellular Bio. 15, 540, inserted a hammerhead ribozyme sequence in the central domain of a VAI RNA.

Pol III-based vectors are described in Stinchcomb et al., WO 95/23225. Another example is provided below.

Example 1: Stromelysin Hammerhead ribozymes

By engineering ribozyme motifs applicant has designed several ribozymes directed against stromelysin mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave stromelysin target sequences in vitro is evaluated.

The ribozymes are tested for function *in vivo* by analyzing stromelysin expression levels. Ribozymes are delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, and/or by expression from DNA/RNA vectors. Stromelysin expression is monitored by biological assays, ELISA, by indirect immunofluoresence, and/or by FACS analysis. Stromelysin mRNA levels are assessed by Northern analysis, RNAse protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of stromelysin activity and/or stromelysin mRNA by more than 50% are identified.

Ribozymes targeting selected regions of mRNA associated with arthritic disease are chosen to cleave the target RNA in a manner which preferably inhibits translation of the RNA. Genes are selected such that inhibition of translation will preferably inhibit cell replication, e.g., by inhibiting production of a necessary protein or prevent production of an undesired protein, e.g., stromelysin. Selection of effective target sites within these critical regions of mRNA may entail testing the accessibility of the target RNA to hybridization with various oligonucleotide probes. These studies can be performed using RNA or DNA probes and assaying accessibility by cleaving the hybrid molecule with RNaseH (see below). Alternatively, such a study can use

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ribozyme probes designed from secondary structure predictions of the mRNAs, and assaying cleavage products by polyacrylamide gel electrophoresis (PAGE), to detect the presence of cleaved and uncleaved molecules.

In addition, potential ribozyme target sites within the rabbit stromelysin mRNA sequence (1795 nucleotides) were located and aligned with the human target sites. Because the rabbit stromelysin mRNA sequence has an 84% sequence identity with the human sequence, many ribozyme target sites are also homologous. Thus, the rabbit has potential as an appropriate animal model in which to test ribozymes that are targeted to human stromelysin but have homologous or nearly homologous cleavage sites on rabbit stromelysin mRNA as well (Tables All-AVI, AVIII & AIX). Thirty of the 316 UH sites in the rabbit sequence are identical with the corresponding site in the human sequence with respect to at least 14 nucleotides surrounding the potential ribozyme cleavage sites. The nucleotide in the RNA substrate that is immediately adjacent (5') to the cleavage site is unpaired in the ribozymesubstrate complex (see Fig. 1) and is consequently not included in the comparison of human and rabbit potential ribozyme sites. In choosing human ribozyme target sites for continued testing, the presence of identical or nearly identical sites in the rabbit sequence is considered.

Example 2: Superior sites

Potential ribozyme target sites were subjected to further analysis using computer folding programs (Mulfold or a Macintosh-based version of the following program, LRNA (Zucker (1989) Science 244:48), to determine if 1) the target site is substantially single-stranded and therefore predicted to be available for interaction with a ribozyme, 2) if a ribozyme designed to that site is predicted to form stem II but is generally devoid of any other intramolecular base pairing, and 3) if the potential ribozyme and the sequence flanking both sides of the cleavage site together are predicted to interact correctly. The sequence of Stem II can be altered to maintain a stem at that position but minimize intramolecular basepairing with the ribozyme's substrate binding arms. Based on these minimal criteria, and including all the sites that are identical in human and rabbit stromelysin mRNA sequence, a subset of 66

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potential superior ribozyme target sites was chosen (as first round targets) for continued analysis. These are SEQ. ID. NOS.: 34, 35, 37, 47, 54, 57, 61, 63, 64, 66, 76, 77, 79, 87, 88, 96, 97, 98, 99, 100, 107, 110, 121, 126, 128, 129, 133, 140, 146, 148, 151, 162, 170, 179, 188, 192, 194, 196, 199, 202, 203, 207, 208, 218, 220, 223, 224, 225, 227, 230, 232, 236, 240, 245, 246, 256, 259, 260, 269, 280, 281, 290, 302, 328, 335 and 353 (see Table AIII).

Example 3: Accessible sites

To determine if any or all of these potential superior sites might be accessible to a ribozyme directed to that site, an RNAse H assay is carried out. Using this assay, the accessibility of a potential ribozyme target site to a DNA oligonucleotide probe can be assessed without having to synthesize a ribozyme to that particular site. If the complementary DNA oligonucleotide is able to hybridize to the potential ribozyme target site then RNAse H, which has the ability to cleave the RNA of a DNA/RNA hybrid, will be able to cleave the target RNA at that particular site. Specific cleavage of the target RNA by RNAse H is an indication that that site is "open" or "accessible" to oligonucleotide binding and thus predicts that the site will also be open for ribozyme binding. By comparing the relative amount of specific RNAse H cleavage products that are generated for each DNA oligonucleotide/site, potential ribozyme sites can be ranked according to accessibility.

To analyze target sites using the RNAse H assay, DNA oligonucleotides (generally 13-15 nucleotides in length) that are complementary to the potential target sites are synthesized. Body-labeled substrate RNAs (either full-length RNAs or ~500-600 nucleotide subfragments of the entire RNA) are prepared by *in vitro* transcription in the presence of a $^{32}\text{P-labeled}$ nucleotide. Unincorporated nucleotides are removed from the $^{32}\text{P-labeled}$ substrate RNA by spin chromatography on a G-50 Sephadex column and used without further purification. To carry out the assay, the $^{32}\text{P-labeled}$ substrate RNA is pre-incubated with the specific DNA oligonucleotide (1 μM and 0.1 μM final concentration) in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM DTT at 37 °C for 5 minutes. An excess of RNAse H (0.8 units/10 μI reaction) is added and the incubation is continued for 10 minutes. The reaction is quenched by the addition of an equal volume of 95% formamide,

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20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. RNAse H-cleaved RNA products are separated from uncleaved RNA on denaturing polyacrylamide gels, visualized by autoradiography and the amount of cleavage product is quantified.

RNAse H analysis on the 66 potential ribozyme sites (round 1) was carried out and those DNA oligonucleotides/sites that supported the most RNAse H cleavage were determined. These assays were carried out using full-length human and rabbit stromelysin RNA as substrates. determined on human stromelysin RNA indicated that 23 of the 66 sites supported a high level of RNAse H cleavage, and an additional 13 supported a moderate level of RNAse H cleavage. Twenty-two sites were chosen from among these two groups for continued study. Two of the criteria used for making this choice were 1) that the particular site supported at least moderate RNAse H cleavage on human stromelysin RNA and 2) that the site have two or fewer nucleotide differences between the rabbit and the human stromelysin sequence. RNAse H accessibility on rabbit stromelysin RNA was determined, but was not used as a specific criteria for these choices. Those DNA oligonucleotides that are not totally complementary to the rabbit sequence may not be good indicators of the relative amount of RNAse H cleavage, possibly because the mismatch leads to less efficient hybridization of the DNA oligonucleotide to the mismatched RNA substrate and therefore less RNAse H cleavage is seen.

Example 4: Analysis of Ribozymes

Ribozymes were then synthesized to 22 sites (Table AV) predicted to be accessible as judged the RNAse H assay. Eleven of these 22 sites are identical to the corresponding rabbit sites. The 22 sites are SEQ. ID, NOS.: 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, 281. The 22 ribozymes were chemically synthesized with recognition arms of either 7 nucleotides or 8 nucleotides, depending on which ribozyme alone and ribozyme-substrate combinations were predicted by the computer folding program (Mulfold) to fold most correctly. After synthesis, ribozymes are either purified by HPLC or get purified.

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These 22 ribozymes were then tested for their ability to cleave both human and rabbit full-length stromelysin RNA. Full-length, body-labeled stromelysin RNA is prepared by in vitro transcription in the presence of [a-32PICTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Assays are performed by prewarming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction is initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that has also been prewarmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 1 μ M and 0.1 μ M ribozyme, i.e., ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Full-length substrate RNA and the specific RNA products generated by ribozyme cleavage are visualized on an autoradiograph of the gel.

.Of the 22 ribozymes tested, 21 were able to cleave human and rabbit substrate RNA in vitro in a site-specific manner. In all cases, RNA cleavage products of the appropriate lengths were visualized. The size of the RNA was judged by comparison to molecular weight standards electrophoresed in adjacent lanes of the gel. The fraction of substrate RNA cleaved during a ribozyme reaction can be used as an assessment of the activity of that ribozyme in vitro. The activity of these 22 ribozymes on full-length substrate RNA ranged from approximately 10% to greater than 95% of the substrate RNA cleaved in the ribozyme cleavage assay using 1 μM ribozyme as described above. A subset of seven of these ribozymes was chosen for continued study. These seven ribozymes (denoted in Table AV) were among those with the highest activity on both human and rabbit stromelysin RNA. Five of these seven sites have sequence identity between human and rabbit stromelysin RNAs for a minimum of 7 nucleotides in both directions flanking the cleavage site. These sites are 883, 947, 1132, 1221 and 1410. and the ribozymes are SEQ. ID. NOS.: 368, 369, 370, 371, 372, 373, and 374.

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Example 5: Arm Length Tests

In order to test the effect of arm length variations on the cleavage activity of a ribozyme to a particular site in vitro, ribozymes to these seven sites were designed that had alterations in the binding arm lengths. For each site, a complete set of ribozymes was synthesized that included ribozymes with binding arms of 6 nucleotides, 7 nucleotides, 8 nucleotides, 10 nucleotides and 12 nucleotides, i.e., 5 ribozymes to each site. These ribozymes were gelpurified after synthesis and tested in ribozyme cleavage assays as described above.

After analysis of the 35 ribozymes, five ribozymes with varied arm lengths to each of these seven sites, it was clear that two ribozymes were the most active *in vitro*. These two ribozymes had seven nucleotide arms directed against human sequence cleavage sites of nucleotide 617 and nucleotide 820. These are referred to as RZ 617H 7/7 and RZ 820H 7/7 denoting the human (H) sequence cleavage site (617 or 820) and the arm length on the 5' and 3' side of the ribozyme molecule.

Example Testing the efficacy of ribozymes in cell culture

The two most active ribozymes in vitro (RZ 617H 7/7 and RZ 820H 7/7) were then tested for their ability to cleave stromelysin mRNA in the cell. Primary cultures of human or rabbit synovial fibroblasts were used in these experiments. For these efficacy tests, ribozymes with 7 nucleotide arms were synthesized with 2' O- methyl modifications on the 5 nucleotides at the 5' end of the molecule and on the 5 nucleotides at the 3' end of the molecule. For comparison, ribozymes to the same sites but with 12 nucleotide arms (RZ 617H 12/12 and RZ 820H 12/12) were also synthesized with the 2' O methyl modifications at the 5 positions at the end of both binding arms. Inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also prepared for use as controls. The catalytic core in the inactive ribozymes is CU<u>U</u>AUGAGGCCGAAAGGCCGA<u>U</u> CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity in vitro when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μM concentration for 1 hour.

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The general assay was as follows: Fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells are maintained in serum-free media. The ribozyme can be applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic lipids (including TransfectamTM, LipofectinTM and LipofectamineTM), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

If a ribozyme is effective in cleaving stromelysin mRNA within a cell, the amount of stromelysin mRNA will be decreased or eliminated. A decrease in the level of cellular stromelysin mRNA, as well as the appearance of the RNA products generated by ribozyme cleavage of the full-length stromelysin mRNA, can be analyzed by methods such as Northern blot analysis, RNAse protection assays and/or primer extension assays. The effect of ribozyme cleavage of cellular stromelysin mRNA on the production of the stromelysin protein can also be meased by a number of assays. These include the ELISA (Enzyme-Linked Immuno Sorbent Assay) and an immunofluorescence assay described below. In addition, functional assays have been published that monitor stromelysin's enzymatic activity by measuring degradation of its primary substrate, proteoglycan.

Example 7: Analysis of Stromelysin Protein

Stromelysin secreted into the media of Interleukin-1α-induced human synovial fibroblasts was measured by ELISA using an antibody that recognizes human stromelysin. Where present, a TransfectamTM-ribozyme complex (0.15 μM ribozyme final concentration) was offered to 2-4 x 10⁵ serum-starved cells for 3 hours prior to induction with Interleukin-1α. The TransfectamTM was prepared according to the manufacturer (Promega Corp.) except that 1:1 (w/w) dioleoyl phosphatidylethanolamine was included. The TransfectamTM-ribozyme complex was prepared in a 5:1 charge ratio. Media was harvested 24 hours after the addition of Interleukin-1α. The control (NO RZ) is TransfectamTM alone applied to the cell. Inactive ribozymes, with 7

nucleotide arms or 12 nucleotide arms have the two inactivating changes to the catalytic core that are described above. Cell samples were prepared in duplicate and the assay was carried out on several dilutions of the conditioned media from each sample. Results of the ELISA are presented below as a percent of stromelysin present vs. the control (NO RZ) which is set at 100%.

		RZ TARGET SITE	
	TREATMENT	617H	820H
	RZ 7/7	06.83	07.05
	RZ 12/12	18.47	33.90
10	INACTIVE RZ 7/7	100	100
	INACTIVE RZ 12/12	100	100
	NO RZ CONTROL	100	100

The results above clearly indicate that treatment with active ribozyme, either RZ 617H 7/7 and RZ 820H 7/7, has a dramatic effect on the amount of stromelysin secreted by the cells. When compared to untreated, control cells or cells treated with inactive ribozymes, the level of stromelysin was decreased by approximately 93%. Ribozymes to the same sites, but synthesized with 12 nucleotide binding arms, were also efficacious, causing a decrease in stromelysin to ~66 to ~81% of the control. In previous in vitro ribozyme cleavage assays, RZ 617H 7/7 and RZ 820H 7/7 had better cleavage activity on full-length RNA substrates than ribozymes with 12 nucleotide arms directed to the same sites (617H 12/12 and RZ 820H 12/12).

25 Example 8: Immunofluorescent Assay

An alternative method of stromelysin detection is to visualize stromelysin protein in the cells by immunofluorescence. For this assay, cells are treated

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with monensin to prevent protein secretion from the cell. The stromelysin retained by the cells after monensin addition can then be visualized by immunofluorescence using either conventional or confocal microscopy. Generally, cells were serum-starved overnight and treated with ribozyme the following day for several hours. Monensin was then added and after ~5-6 hours, monensin-treated cells were fixed and permeabilized by standard methods and incubated with an antibody recognizing human stromelysin. Following an additional incubation period with a secondary antibody that is conjugated to a fluorophore, the cells were observed by microscopy. A decrease in the amount of fluorescence in ribozyme-treated cells, compared to cells treated with inactive ribozymes or media alone, indicates that the level of stromelysin protein has been decreased due to ribozyme treatment.

As visualized by the immunofluorescence technique described above, treatment of human synovial fibroblasts with either RZ 617H 7/7 or RZ 820H 7/7 (final concentrations of 1.5 μ M free ribozyme or 0.15 μ M ribozyme complexed with TransfectamTM resulted in a significant decrease in fluorescence, and therefore stromelysin protein, when compared with controls. Controls consisted of tre-ling with media or TransfectamTM alone. Treatment of the cells with the corresponding inactive ribozymes with two inactivating changes in the catalytic core resulted in immunofluorescence similar to the controls without ribozyme treatment.

Rabbit synovial fibroblasts were also treated with RZ 617H 7/7 or RZ 820H 7/7, as well as with the two corresponding ribozymes (RZ 617R 7/7 or RZ 820R 7/7) that each have the appropriate one nucleotide change to make them completely complementary to the rabbit target sequence. Relative to controls that had no ribozyme treatment, immunofluorescence in Interleukin-1 α -induced rabbit synovial fibroblasts was visibly decreased by treatment with these four ribozymes, whether specific for rabbit or human mRNA sequence. For the immunofluorescence study in rabbit synovial fibroblasts, the antibody to human stromelysin was used.

Example 9: Ribozyme Cleavage of Cellular RNA

The following method was used in this example.

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Primer extension assay:

The primer extension assay was used to detect full-length RNA as well as the 3' ribozyme cleavage products of the RNA of interest. The method involves synthesizing a DNA primer (generally ~20 nucleotides in length) that can hybridize to a position on the RNA that is downstream (3') of the putative ribozyme cleavage site. Before use, the primer was labeled at the 5' end with 32P[ATP] using T4 polynucleotide kinase and purified from a gel. The labeled primer was then incubated with a population of nucleic acid isolated from a cellular lysate by standard procedures. The reaction buffer was 50 mM Tris-HCI, pH 8.3, 3 mM MgCl₂, 20 mM KCI, and 10 mM DTT. A 30 minute extension reaction follows, in which all DNA primers that have hybridized to the RNA were substrates for reverse transcriptase, an enzyme that will add nucleotides to the 3' end of the DNA primer using the RNA as a template. Reverse transcriptase was obtained from Life Technologies and is used essentially as suggested by the manufacturer. Optimally, reverse transcriptase will extend the DNA primer, forming cDNA, until the end of the RNA substrate is reached. Thus, for ribozyme-cleaved RNA substrates, the cDNA product will be shorter than the resulting cDNA product of a full-length. or uncleaved RNA substrate. The differences in size of the 32P-labeled cDNAs produced by extension can then be discriminated by electrophoresis on a denaturing polyacrylamide gel and visualized by autoradiography.

Strong secondary structure in the RNA substrate can, however, lead to premature stops by reverse transcriptase. This background of shorter cDNAs is generally not a problem unless one of these prematurely terminated products electrophoreses in the expected position of the ribozyme-cleavage product of interest. Thus, 3' cleavage products are easily identified based on their expected size and their absence from control lanes. Strong stops due to secondary structure in the RNA do, however, cause problems in trying to quantify the total full-length and cleaved RNA present. For this reason, only the relative amount of cleavage can easily be determined.

The primer extension assay was carried out on RNA isolated from cells that had been treated with Transfectam™-complexed RZ 617H 7/7, RZ 820H 7/7, RZ 617H 12/12 and RZ 820H 12/12. Control cells had been treated with

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TransfectamTM alone. Primer extensions on RNA from cells treated with the TransfectamTM complexes of the inactive versions of these four ribozymes were also prepared. The 20 nucleotide primer sequence is 5' AATGAAAACGAGGTCCTTGC 3' and it is complementary to a region about 285 nucleotides downstream of ribozyme site 820. For ribozymes to site 617, the cDNA length for the 3' cleavage product is 488 nucleotides, for 820 the cDNA product is 285 nucleotides. Full-length cDNA will be 1105 nucleotides in length. Where present, 1 ml of 0.15 μM ribozyme was offered to ~2-3 x 10⁵ serum-starved human synovial fibroblasts. After 3 hours, 20 units/ml Interleukin-1α was added to the cells and the incubation continued for 24 hours.

32P-labeled cDNAs of the correct sizes for the 3' products were clearly visible in lanes that contained RNA from cells that had been treated with active ribozymes to sites 617 and 820. Ribozymes with 7 nucleotide arms were judged to be more active than ribozymes with 12 nucleotide arms by comparison of the relative amount of 3' cleavage product visible. This correlates well with the data obtained by ELISA analysis of the conditioned media from these same samples: In addition, no cDNAs corresponding to the 3' cleavage products were visible following treatment of the cells with any of the inactive ribozymes.

To insure that ribozyme cleavage of the RNA substrate was not occurring during the preparation of the cellular RNA or during the primer extension reaction itself, several controls have been carried out. One control was to add body-labeled stromelysin RNA, prepared by *in vitro* transcription, to the cellular lysate. This lysate was then subjected to the typical RNA preparation and primer extension analysis except that non-radioactive primer was used. If ribozymes that are present in the cell at the time of cell lysis are active under any of the conditions during the subsequent analysis, the added, body-labeled stromelysin RNA will become cleaved. This, however, is not the case. Only full-length RNA was visible by gel analysis, no ribozyme cleavage products were present. This is evidence that the cleavage products detected in RNA from ribozyme-treated cells resulted from ribozyme cleavage in the cell, and not during the subsequent analysis.

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Example 10: RNAse Protection Assay

By RNAse protection analysis, both the 3' and the 5' products generated by ribozyme cleavage of the substrate RNA in a cell can be identified. The RNAse protection assay is carried out essentially as described in the protocol provided with the Lysate Ribonuclease Protection Kit (United States Biochemical Corp.) The probe for RNAse protection is an RNA that is complementary to the sequence surrounding the ribozyme cleavage site. This "antisense" probe RNA is transcribed in vitro from a template prepared by the polymerase chain reaction in which the 5' primer was a DNA oligonucleotide containing the T7 promoter sequence. The probe RNA is body labeled during transcription by including ³²P[CTP] in the reaction and purified away from unincorporated nucleotide triphosphates by chromatography on G-50 Sephadex. The probe RNA (100,000 to 250,000 cpms) is allowed to hybridize overnight at 37°C to the RNA from a cellular lysate or to RNA purified from a cell lysate. After hybridization, RNAse T_1 and RNAse A are added to degrade all single-stranded RNA and the resulting products are analyzed by gel electrophoresis and autoradiography. By this analysis, full-length, uncleaved target RN/2 will protect the full-length probe. For ribozyme-cleaved target RNAs, only a portion of the probe will be protected from RNAse digestion because the cleavage event has occurred in the region to which the probe binds. This results in two protected probe fragments whose size reflects the position at which ribozyme cleavage occurs and whose sizes add up to the size of the full-length protected probe.

RNAse protection analysis was carried out on cellular RNA isolated from rabbit synovial fibroblasts that had been treated either with active or inactive ribozyme. The ribozymes tested had 7 nucleotide arms specific to the rabbit sequence but corresponding to human ribozyme sites 617 and 820 (i.e. RZ 617R 7/7, RZ 820R 7/7). The inactive ribozymes to the same sites also had 7 nucleotide arms and included the two inactivating changes described above. The inactive ribozymes were not active on full-length rabbit stromelysin RNA in a typical 1 hour ribozyme cleavage reaction in vitro at a concentration of 1 μM. For all samples, one ml of 0.15 μM ribozyme was administered as a TransfectamTM complex to serum-starved cells. Addition of Interleukin-1α followed 3 hours later and cells were harvested after 24 hours. For samples

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from cells treated with either active ribozyme tested, the appropriately-sized probe fragments representing ribozyme cleavage products were visible. For site 617, two fragments corresponding to 125 and 297 nucleotides were present, for site 820 the two fragments were 328 and 94 nucleotides in length. No protected probe fragments representing RNA cleavage products were visible in RNA samples from cells that not been treated with any ribozyme, or in cells that had received the inactive ribozymes. Full-length protected probe (422 nucleotides in length) was however visible, indicating the presence of full-length, uncleaved stromelysin RNA in these samples.

10 Delivery of Free and Transfectam-Complexed Ribozymes to Fibroblasts

Ribozymes can be delivered to fibroblasts complexed to a cationic lipid or in free form. To deliver free ribozyme, an appropriate dilution of stock ribozyme (final concentration is usually 1.5 μ M) is made in serum-free medium; if a radioactive tracer is to be used (i.e., 32 P), the specific activity of the ribozyme is adjusted to 800-1200 cpm/pmol. To deliver ribozyme complexed with the cationic lipid Transfectam, the lipid is first prepared as a stock solution containing 1/1 (w/w) dioleoylphic-sphatidylcholine (DOPE). Ribozyme is mixed with the Transfectam/DOPE mixture at a 1/5 (RZ/TF) charge ratio; for a 36-mer ribozyme, this is a 45-fold molar excess of Transfectam (Transfectam has 4 positive charges per molecule). After a 10 min incubation at room temperature, the mixture is diluted and applied to cells, generally at a ribozyme concentration of 0.15 μ M. For 32 P experiments, the specific activity of the ribozyme is the same as for the free ribozyme experiments.

After 24 hour, about 30% of the offered Transfectam-ribozyme cpm's are cell-associated (in a nuclease-resistant manner). Of this, about 10-15% of the cpm's represent intact ribozyme; this is about 20-25 million ribozymes per cell. For the free ribozyme, about 0.6% of the offered dose is cell-associated after 24 hours. Of this, about 10-15% is intact; this is about 0.6-0.8 million ribozymes per cell.

30 Example 11: In vitro cleavage of stromelysin mRNA by HH ribozymes

In order to screen for additional HH ribozyme cleavage sites, ribozymes, targeted against some of the sites listed in example 2 and Table 3, were

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synthesized. These ribozymes were extensively modified such that: 5' terminal nucleotides contain phosphorothioate substitutions; except for five ribose residues in the catalytic core, all the other 2'-hydroxyl groups within the ribozyme were substituted with either 2'-O-methyl groups or 2'-C-allyl modifications. The aforementioned modifications are meant to be non-limiting modifications. Those skilled in the art will recognize that other embodiments can be readily generated using the techniques known in the art.

These ribozymes were tested for their ability to cleave RNA substrates in vitro. Referring to Fig. 7, in vitro RNA cleavage by HH ribozymes targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 (SEQ. ID. NOS. 35, 98, 202, 263, 279, 281 and 292 respectively) was assayed at 37°C. Substrate RNAs were 5' end-labeled using [γ-32P]ATP and T4 polynucleotide kinase enzyme. In a standard cleavage reaction under "ribozyme excess" conditions, ~1 nM substrate RNA and 40 nM ribozyme were denatured separately by heating to 90°C for 2 min followed by snap cooling on ice for 10 min. The substrate and the ribozyme reaction mixtures were renatured in a buffer containing 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂ at 37°C for 10 min. Cleavage reaction was initiated by mixing the ribozyme and the substrat. FiNA and incubating at 37°C. Aliquots of 5 μl were taken at regular intervals of time and the reaction quenched by mixing with an equal volume of formamide stop mix. The samples were resolved on a 20% polyacrylamide/urea gel.

A plot of percent RNA substrate cleaved as a function of time is shown in Fig. 7. The plot shows that all six HH ribozymes cleaved the target RNA efficiently. Some HH ribozymes were, however, more efficient than others (e.g., 1049HH cleaves faster than 1366HH).

Ribozyme Efficacy Assay in Cultured HS-27 Cells (Used in the Following Examples):

Ribozymes were assayed on either human foreskin fibroblasts(HS-27) cell line or primary human synovial fibroblasts (HSF). All cells were plated the day before the assay in media containing 10% fetal bovine serum in 24 well plates at a density of 5x10⁴ cells/well. At 24 hours after plating, the media was removed from the wells and the monolayers were washed with Dulbeccos

phosphate buffered saline (PBS). The cells were serum starved for 24 h by incubating the cells in media containing 0.5% fetal bovine serum (FBS: 1 ml/well). Ribozyme/lipid complexes were prepared as follows: Ribozymes and LipofectAMINE were diluted separately in serum-free DMEM plus 20 mM Hepes pH 7.3 to 2X final concentration, then equal volumes were combined. vortexed and incubated at 37°C for 15 minutes. The charge ratio of LipofectAmine: ribozyme was 3:1. Cells were washed twice with PBS containing Ca²⁺ and Mg²⁺. Cells were then treated the ribozyme/lipid complexes and incubated at 37°C for 1.5 hours. FBS was then added to a final concentration of 10%. Two hours after FBS addition, the ribozyme containing solution was removed and 0.5 ml DMEM containing 50 u/ml IL-1. 10% FBS, 20 mM Hepes pH 7.3 added. Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al.) supra) to measure stromelysin expression.

Example 12: Ribozyme-Mediated Inhibition of Stromelysin Expression in human fibroblast cells

20 Referring to Figs. 8 through 13, HH ribozymes, targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 within human stromelysin-1 mRNA, were transfected into HS-27 fibroblast or HSF cell line as described above. Catalytically inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also synthesized for use as controls. The catalytic 25 core in the inactive ribozymes was CUUAUGAGGCCGAAAGGCCGAU versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity in vitro when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μM concentration for 1 hour. Levels of stromelysin protein were measured using a sensitive ELISA 30 protocol as described above. + IL-1 in the figures mean that cells were treated with IL-1 to induce the expression of stromelysin expression. -IL-1 means that the cells were not treated. Figs. 8 through 13 show the dramatic reduction in the levels of stromelysin protein expressed in cells that were transfected with active HH ribozymes. This decrease in the level of

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stromelysin production is over and above some non-specific inhibition seen in cells that were transfected with catalytically inactive ribozymes. There is on an average a greater than 50% inhibition in stromelysin production (in cells transfected with active HH ribozymes) when compared with control cells that were transfected with inactive ribozymes. These results suggest that the reduction in stromelysin production in HS-27 cells is mediated by sequence-specific cleavage of human stromelysin-1 mRNA by catalytically active HH ribozymes. Reduction in stromelysin protein production in cells transfected with catalytically inactive ribozymes may be due to some "antisense effect" caused by binding of the inactive ribozyme to the target RNA and physically preventing translation.

Example 13: Ribozyme-mediated inhibition of stromelysin expression in Rabbit Knee

In order to extend the ribozyme efficacy in cell culture, applicant has chosen to use rabbit knee as a reasonable animal model to study ribozyme-mediated inhibition of rabbit stromelysin protein expression. Applicant selected a HH ribozyme (1049HH), targeted to site 1049 with human stromelysin-1 mRNA, for animal studies because site 1049 is 100% identical to site 1060 (Tables AllI and AVI) within rabbit stromelysin mRNA. This has enabled applicant to compare the efficacy of the same ribozyme in human as well as in rabbit systems.

Male New Zealand White Rabbits (3-4 Kg) were anaesthetized with ketamine-HCl/xylazine and injected intra-articularly (I.T.) in both knees with 100 μg ribozyme (e.g., SEQ. ID. NO. 202) in 0.5 ml phosphate buffered saline (PBS) or PBS alone (Controls). The IL-1 (human recombinant IL-1α, 25 ng) was administered I.T., 24 hours following the ribozyme administration. Each rabbit received IL-1 in one knee and PBS alone in the other. The synovium was harvested 6 hours post IL-1 infusion, snap frozen in liquid nitrogen, and stored at -80°C. Total RNA is extracted with TRIzol reagent (GIBCO BRL, Gaithersburg, MD), and was analyzed by Northern-blot analysis and/or RNase-protection assay. Briefly, 0.5 μg cellular RNA was separated on 1.0 % agarose/formaldehyde gel and transferred to Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, CA) by capillary transfer for ~16 hours. The blots were

baked for two hours and then pre-hybridized for 2 hours at 65°C in 10 ml Church hybridization buffer (7 % SDS, 500 mM phosphate, 1 mM EDTA, 1% Bovine Serum Albumin). The blots were hybridized at 65°C for ~16 hours with 106 cpm/ml of full length 32P-labeled complementary RNA (cRNA) probes to rabbit stromelysin mRNA (cRNA added to the pre-hybridization buffer along with 100 µl 10mg/ml salmon sperm DNA). The blot was rinsed once with 5% SDS, 25 mM phosphate, 1 mM EDTA and 0.5% BSA for 10 min at room temperature. This was followed by two washes (10 min each wash) with the same buffer at 65°C, which was then followed by two washes (10 min each wash) at 65°C with 1% SDS, 25 mM phosphate and 1 mM EDTA. The blot was autoradiographed. The blot was reprobed with a 100 nt cRNA probe to 18S rRNA as described above. Following autoradiography, the stromelysin expression was quantified on a scanning densitometer, which is followed by normalization of the data to the 18S rRNA band intensities.

As shown in Figs. 14-16, catalytically active 1049HH ribozyme mediates a decrease in the expression of stromelysin expression in rabbit knees. The inhibition appears to be sequence-specific and ranges from 50-70%.

Example 14: Phosphorothioate-substituted Ribozymes inhibit stromelysiruexpression in Rabbit Knee

Ribozymes containing four phosphorothioate linkages at the 5' termini enhance ribozyme efficacy in mammalian cells. Referring to Fig. 17, applicant has designed and synthesized hammerhead ribozymes targeted to site 1049 within stromelysin RNA, wherein, the ribozymes contain five phosphorothioate linkages at their 5' and 3' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 30 nucleotide positions, 2'-C-allyl substitution at U4 position and 2'-OH at five positions (Fig 17A). As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. The 1049 U4-C-allyl P=S active ribozyme shows greater than 50 % reduction in the level of stromelysin RNA in rabbit knee. Catalytically inactive version of the 1049 U4-C-allyl P=S ribozyme shows ~30% reduction in the level of stromelysin RNA.

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Referring to Fig. 18, applicant has also designed and synthesized hammerhead ribozymes targeted to three distinct sites within stromelysin RNA, wherein, the ribozymes contain four phosphorothioate linkages at their 5' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 29 nucleotide positions, 2'-amino substitutions at U4 and U7 positions and 2'-OH at five positions. As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. As shown in Figures 18-21, ribozymes targeted to sites 1049, 1363 and 1366 are all efficacious in rabbit knee. All three ribozymes decreased the level of stromelysin RNA in rabbit knee by about 50 %.

Sequences and chemical modifications described in figures 17 and 18 are meant to be non-limiting examples. Those skilled in the art will recognize that similar embodiments with other ribozymes and ribozymes containing other chemical modifications can be readily generated using techniques known in the art and are within the scope of the present invention.

Applicant has shown that chemical modifications, such as 6-methyl U and abasic (nucleotide containing no base) moieties can be substituted at certain positions within the ribozyme, for example U4 and U7 positions, without significantly effecting the catalytic activity of the ribozyme. Similarly, 3'-3' linked abasic inverted ribose moieties can be used to protect the 3' ends of ribozymes in place of an inverted T without effecting the activity of the ribozyme.

B7-1, B7-2, B7-3 and CD40 are attractive ribozyme targets by several criteria. The molecular mechanism of T cell activation is well-established. Efficacy can be tested in well-defined and predictive animal models. The clinical end-point of graft rejection is clear. Since delivery would be *ex vivo*, treatment of the correct cell population would be assured. Finally, the disease condition is serious and current therapies are inadequate. Whereas protein-based therapies would induce anergy against all antigens encountered during the several week treatment period, *ex vivo* ribozyme therapy provides a direct and elegant approach to truly donor-specific anergy.

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Similarly, autoimmune diseases and allergies can be prevented or treated by reversing the devastating course of immune response to self-antigens. Specifically, nucleic acids of this inventions can dampen the response to naturally occuring antigens.

5 Example 15; B7-1, B7-2, B7-3 and/or CD40 Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against B7-1, B7-2, B7-3 and/or CD40 encoded mRNA sequences. These ribozymes were synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences in vitro was evaluated.

Several common human cell lines are available that can be induced to express endogenous B7-1, B7-2, B7-3 and/or CD40. Alternatively, murine splenic cells can be isolated and induced, to express B7-1 or B7-2, with IL-4 or recombinant CD40 ligand. B7-1 and B7-2 can be detected easily with monoclonal antibodies. Use of appropriate flourescent reagents and flourescence-activated cell-sorting (FACS) will permit direct quantitation of surface B7-1 and B7-2 on a cell-by-cell basis. Active ribozymes are expected to directly reduce B7-1 or B7-2 expression. Ribozymes targeted to CD40 would prevent induction of B7-2 by CD40 ligand.

Several animal models of transplantation are available – Mouse, rat, Porcine model (Fodor et al., 1994, *Proc. Natl. Acad. Sci. USA* 91, 11153); or Baboon (reviewed by Nowak, 1994 *Science* 266, 1148). B7-1, B7-2, B7-3 and/or CD40 protein levels can be measured clinically or experimentally by FACS analysis. B7-1, B7-2, B7-3 and/or CD40 encoded mRNA levels will be assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of B7-1, B7-2, B7-3 and/or CD40 activity and/or B7-1, B7-2, B7-3 and/or CD40 protein encoding mRNAs by more than 20% *in vitro* will be identified.

Several animals models of autoimmune disorders are available—allergic encephalomyelitis (EAE) in Lewis rats (Carlson et al., 1993 Ann. N.Y. Acad. Sci. 685, 86); animal models of multiple sclerosis (Wekerle et al., 1994 Ann.

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Neurol. 36, s47) and rheumatoid arthritis (van Laar et al., 1994 Chem. Immunol. 58, 206).

Several animal models of allergy are available and are reviewed by Kemeny and Diaz-Sanchez, 1990, Clin. Exp. Immunol. 82, 423 and Pretolani et al., 1994 Ann. N.Y.Acad. Sci. 725, 247).

RNA ribozymes and/or genes encoding them will be delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery or plasmid vector delivery in these animal model experiments (see above). One dose of a ribozyme vector that constitutively expresses the ribozyme or one or more doses of a stable anti-B7-1, B7-2, B7-3 and/or CD40 ribozymes or a transiently expressing ribozyme vector to donor APC, followed by infusion into the recipient may reduce the incidence of graft rejection. Alternatively, graft tissues may be treated as described above prior to transplantation.

15 Example 16: Synthesis of 6-methyl-uridine phosphoramidite

Referring to Figure 30, the suspension of 6-methyl-uracil (2.77g, 21.96 mmol) in the mixture of hexamethyldisilazane (50mL) and dry pyridine (50mL) was refluxed for three hours. The resulting clear solution of trimethylsilyl derivative of 6-methyl uracyl was evaporated to dryness and coevaporated 2 times with dry toluene to remove traces of pyridine. To the solution of the resulting clear oil, in dry acetonitrile, 1-O-acetyl-2',3',5'-tri-O-benzoyl-b-D-ribose (10.1g, 20 mmol) was added and the reaction mixture was cooled to 0°C. To the above stirred solution, trimethylsilyl trifluoromethanesulfonate (4.35 mL, 24 mmol) was added dropwise and the reaction mixture was stirred for 1.5 h at 0°C and then 1h at room temperature. After that the reaction mixture was diluted with dichloromethane washed with saturated sodium bicarbonate and brine. The organic layer was evaporated and the residue was purified by flash chromatography on silica gel with ethylacetate-hexane (2:1) mixture as an eluent to give 9.5g (83%) of the compound 2 and 0.8g of the corresponding N1,N3-bis-derivative.

To the cooled (-10°C) solution of the compound (4.2g, 7.36 mmol) in the mixture of pyridine (60 mL) and methanol (10 mL) ice-cooled 2M aqueous

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solution of sodium hydroxide (16 mL) was added with constant stirring. The reaction mixture was stirred at -10°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py+). The resin was filtered off and washed with a 200 mL mixture of H2O - Pyridine (4:1). The combined "mother liquor" and the washings were evaporated to dryness and dried by multiple coevaporation with dry pyridine. The residue was redissolved in dry pyridine and then mixed with dimethoxytrityl chloride (2.99g, 8.03 mmol). The reaction mixture was left ovemight at room temperature. Reaction was-quenched with methanol (25 mL) and the mixture was evaporated. The residue was dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (2% to 5%) in CH2Cl2 as eluent to give 3.4g (83%) of the compound 6.

15 Example 17: Synthesis of 6-methyl-cytidine phosphoramidite

Triethylamine (13.4 ml, 100 mmol) was added dropwise to a stirred icecooled mixture of 1,2,4-triazole (6.22g, 90 mmol) and phosphorous oxychloride (1.89 ml, 20 mmol) in 50 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3',5'-tri-O-Benzoyl-6-methyl uridine (5.7g, 10 mmol) in 30 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 4 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 100 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH4OH overnight. The solvents were removed in vacuo. The residue was dissolved in the in the mixture of pyridine (60 mL) and methanol (10 mL), cooled to -15°C and ice-cooled 2M ag solution of sodium hydroxide was added under stirring. The reaction mixture was stirred at -10 to -15°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py+). The resin was filtered off and washed with 200.mL of the mixture H₂O - Py (4:1). The combined mother liquor and washings were evaporated to dryness. The residue was crystallized from aq methanol to give 1.6g (62%) of 6-methyl cytidine.

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To the solution of 6-methyl cytidine (1.4g, 5.44 mmol) in dry pyridine 3.11 mL of trimethylchlorosilane was added and the reaction mixture was stirred for 2 hours at room temperature. Then acetic anhydride (0.51 mL, 5.44 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. TLC showed disappearance of the starting material and the reaction was quenched with MeOH (20 mL), ice-cooled and treated with water (20 mL, 1 hour). The solvents wee removed in vacuo and the residue was dried by four coevaporations with dry pyridine. Finally it was redissolved in dry pyridine and dimethoxytrityl chloride (2.2 g, 6.52 mmol) was added. The reaction mixture was stirred overnight at room temperature and quenched with MeOH (20 mL). The solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate and brine. The organic layer was separated and evaporated and the residue was purified by flash chromatography on silica gel with the gradient of MeOH in methylene chloride (3% to 5%) to give 2.4 g (74%) of the compound (4).

Example 18: Synthesis of 6-aza-uridine and 6-aza-cytidine

To the solution of 6-aza uridine (5g, 20.39 mmol) in dry pyridine dimethoxytrityl chloride (8.29g, 24.47 mmol) was added and the reaction mixture was left overnight at room temperature. Then it was quenched with methanol (50 mL) and the solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate and brine. The organic layer was separated and evaporated to dryness. The residue was additionally dried by multiple coevaporations with dry pyridine and finally dissolved in dry pyridine. Acetic anhydride (4.43 mL, 46.7 mmol) was added to the above solution and the reaction mixture was left for 3 hours at room temperature. Then it was quenched with methanol and worked-up as above. The residue was purified by flash chromatography on silics gel using mixture of 2% of MeOH in methylene chloride as an eluent to give 9.6g (75%) of the compound.

Triethylamine (23.7 ml, 170.4 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (10.6g, 153.36 mmol) and phosphorous oxychloride (3.22 ml, 34.08 mmol) in 100 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3'-di-O-Acetyl-5'-O-Dimethoxytrityl-6-

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aza Uridine (7.13g, 11.36 mmol) in 40 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 6 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 150 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH4OH for 20 hours at room temperature. The solvents were removed in vacuo. The residue was purified by flash chromatigraphy on silica gel using linear gradient of MeOH (4% to 10%) in methylene chloride as an eluent to give 3.1g (50%) of azacytidine.

To the stirred solution of 5'-O-Dimethoxytrityl-6-aza cytidine (3g, 5.53 mmol) in anhydrous pyridine trimethylchloro silane (2.41 mL, 19 mmol) was added and the reaction mixture was left for 4 hours at room temperature. Then acetic anhydride (0.63 mL, 6.64 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. After that it was quenched with MeOH (15 mL) and the solvents were removed in vacuo. The residue was treated with 1M solution of tetrabutylammonium fluoride in THF (20°, 30 min) and evaporated to dryness. The remaining oil was dissolved in methylene chloride, washed with saturated aq sodium bicarbonate and water. The separated organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 4% MeOH in methylene chloride as an eluent to give 2.9g (89.8%) of the compound.

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO3 (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO3. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

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General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL). Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Example 19: RNA cleavage activity of HHA ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site A (see Fig. 31) were synthesized using solid-phase synthesis, as described above. U4 position was modified with 6-methyl-uridine.

RNA cleavage assay in vitro:

Substrate RNA is 5' end-labeled using [γ-32P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl2. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 32, hammerhead ribozymes containing 6-methyl-uridine modification at U4 position cleave the target RNA efficiently.

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Example 20: RNA cleavage activity of HHB ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site B (see Fig. 33) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 34, hammerhead ribozymes containing 6-methyl-uridine-modification at U4 and U7 positions cleave the target RNA efficiently.

Example 21: RNA cleavage activity of HHC ribozyme substituted with 6methyl-Uridine

Hammerhead ribozymes targeted to site C (see Fig. 35) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 36, hammerhead ribozymes containing 6-methyl-uridine modification at U4 positions cleave the target RNA efficiently.

Sequences listed in Figure 23, 31, 33, 35, and others and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 22: Inhibition of Rat smooth muscle cell proliferation by 6-methyl-U substituted ribozyme HHA.

Hammerhead ribozyme (HHA) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of c-myb protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown *et al.*, 1992 *J. Biol. Chem.* 267, 4625).

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The ribozymes that cleaved site A within c-myb RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb et al., supra). HHA ribozymes were complexed with lipids and delivered into rat smooth muscle cells. Serum-starved cells were stimulated as described by Stinchcomb et al., supra. Briefly, serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 μM bromodeoxyuridine (BrdU) was added. In some wells, FBS was omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H2O2 in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100 (Ribozyme - 0% serum)/(Control - 0% serum).

20 Referring to Figure 37, active ribozymes substituted with 6-methyl-U at position 4 of HHA were successful in inhibiting rat smooth muscle cell proliferation. A catalytically inactive ribozyme (inactive HHA), which has two base substitutions within the core (these mutations inactivate a hammerhead ribozyme; Stinchcomb et al., supra), does not significantly inhibit rat smooth muscle cell proliferation.

25 Example 23: Inhibition of stromelysin production in human synovial fibroblast cells by 6-methyl-U substituted ribozyme HHC.

Hammerhead ribozyme (HHC) is targeted to a unique site (site C) within stromelysin mRNA.

The general assay was as described (Draper et al., supra). Briefly, 30 fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells were maintained in serum-free media. The ribozyme were applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic

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lipids (including TransfectamTM, LipofectinTM and LipofectamineTM), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al., supra) to measure stromelysin expression.

Referring to Figure 38. HHC ribozyme containing 6-methyl-U modification, caused a significant reduction in the level of stromelysin protein production. Catalytically inactive HHC had no significant effect on the protein level.

Example 24: Synthesis of pyridin-2(4)-one nucleoside 3'-phosphoramidites

General procedure for the preparation of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2(4)-pyridones (3) and (9)

Referring to Figure 39, 2- or 4-hydroxypyridine (1) or (8) (2.09 g, 22 20 mmol), 1-O-acetyl-2,3,5-tri-O-benzoyl-B-D-ribofuranose (2) (10.08 g, 20 mmol) and BSA (5.5 ml, 22 mmol) were dissolved in dry acetonitrile (100 ml) under argon at 70°C (oil bath) and the mixture stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (TMSTfl) (5.5 ml, 28.5 mmol) was added and the mixture was stirred for an additional hour for 1 or four hours for 8. The mixture 25 was then cooled to room temperature (RT) followed by dilution, with CHCl3 (200 ml), and extraction, with sat. aq. NaHCO3 solution. The organic layer was washed with brine, dried (Na₂SO₄) and evaporated to dryness in vacuo. The residue was chromatographed on the column of silica gel; 1-5% gradient of methanol in dichloromethane was used for purification of 3 (98% yield) and 30 2-10% gradient of methanol in dichloromethane for purification of 9 (84% yield).

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1-(β-D-Ribofuranosyl)-2(4)-pyridones (4) and (10)

3 or 9 (18 mmol) was dissolved in 0.3M NaOCH₃ (150 ml) and the solution was stirred at RT for 1 hour. The mixture was then neutralized, with Dowex 50WX8 (Py+), the ion-exchanger was filtered off and the filtrate was concentrated to a syrup *in vacuo*. The residue was dissolved in water (100 ml) and the solution was washed with chloroform (2 x 50 ml) and ether (2 x50 ml). The aqueous layer was evaporated to dryness and the residue was then crystallized from ethyl acetate (3.9 g, 91% 4; Niedballa *et al.*, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.; J. Wiley & Sons, Inc.; New York, 1978, p 481-484); 10 (Niedballa and Vorbrüggen, *J. Org. Chem.* 1974, 39, 3668-3671) was crystallized from ethanol (3.6 g, 84%).

1-(2-O-TBDMSi-5-O-DMT-β-D-ribofuranosyl)-2(4)-pyridones

4 or 10 was 5'-O-dimethoxytritylated according to the standard procedure (see Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 5 in 76% yield and pyridin-4-one derivative in 67% yield in the form of yellowish foams after silica gel column chromatography (0.5-10% gradient of methanol in dichloromethane). These compounds were treated with t-butyldimethylsilyl chloride under the conditions described by Hakimelahi et al., Can. J. Chem. 1982, 60, 1106-1113, and the reaction mixtures were purified by the silica gel column chromatography (20-50% gradient of ethyl acetate in hexanes) to enable faster moving 2'-O-TBDMSi isomers (68.5% and 55%, respectively) as colorless foams.

1-[2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite]-2(4)-pyridones (7) and (11)

- 1-(2-O-TBDMS-5-O-DMT-β-D-ribofuranosyl)-2(4)-pyridones were phosphitylated under conditions described by Tuschl *et al.*, *Biochemistry* 1993, 32, 11658-11668, and the products were isolated by silica gel column chromatography using 15-50% gradient of ethyl acetate in hexanes (1% Et₃N) for 7 (89% yield) and dichloromethane (1% Et₃N) for 11 (94% yield).
- Phosphoramidites 7 and 11 were incorporated into ribozymes and substrates using the method of synthesis, deprotection, purification and testing

previously described (Wincott et al., 1995 supra). The average stepwise coupling yields were ~98 %.

Example 25: Synthesis of 2-*O-t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N.N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl-β-D-ribofuranose (8) phosphoramidites

<u>5-O-t-Butyldiphenylsilyl-2.3-O-isopropylidene-1-deoxy-1-phenyl-β-D-ribofuranose (3)</u>

Referring to Figure 40, compound 3 was prepared using the procedure analogous to that described by Czemecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using the more acid resistant *t*-butyldiphenylsilyl group for 5-*O*-protection, instead of *t*-butyldimethylsilyl.

1-Deoxy-1-phenyl-β-D-ribofuranose (5)

Compound 3 (1 g, 2.05 mmol) was dissolved in THF (20 ml) and the solution was mixed with 1M TBAF in THF (3 ml, 3 mmol). The reaction mixture was stirred at RT for 30 min followed by evaporation into a syrup. The residue was applied on to a silica gel column and eluted with hexanes followed by 5-70% gradient of ethyl acetate in hexanes. The 5-O-desilylated product was obtained as a colorless foam (0.62 g, 88% yield). This material was dissolved in 70% acetic acid and heated at 100°C (oil bath) for 30 min. Evaporation to dryness under reduced pressure and crystallization of the residual syrup from toluene resulted in 5 (0.49 g, 94% yield), mp 120-121°C.

2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-1-deoxy-1-phenyl-β-D-ribofuranose (7)

Compound 5 (770 mg, 3.66 mmol) was 5-O-dimethoxytritylated according to the standard procedure (Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 1.4 g (75% yield) of 5-O-dimethoxytrityl derivative as a yellowish foam, following silica gel column chromatography (0.5-2% gradient of methanol in dichloromethane). This material was treated with t-butyldimethylsilyl chloride under the conditions described by Hakimelahi et al., Can. J. Chem. 1982, 60, 1106-1113, and the reaction mixture

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was purified by silica gel column chromatography (2-10% gradient of ethyl acetate in hexanes) to afford a slower moving 2'-O-TBDMSi isomer 7 (0.6 g, 35% yield) as a colorless foam. The faster migrating 3'-O-TBDMSi isomer 6 was also isolated (0.55 g, 32% yield).

2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-phenyl-β-D-ribofuranose (8)

Compound 7 (0.87 g, 1.39 mmol) was phosphitylated under conditions described by Tuschl *et al.*, *supra* and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et₃N) for elution (0.85 g, 74% yield).

Example 26: Synthesis of pseudouridine, 3-methyluridine and 2,4,6-trimethoxy benzene nucleoside phosphoramidites

Starting with a pseudo uridine, 3-methyluridine or 2,4,6-trimethoxy benzene nucleoside (Gasparutto et al., Nucleic Acid Res. 1992 20, 5159-5166; Kalvoda and Farkas, Nucleic Acid Chemistry, Part 1, Townsend, L.B. and Tipson, R.S., Ed. J. Wilk Sons, Inc.; New York, 1978, p 481-484), phosphoramidites can be prepared by standard protocols described below (Figure 41).

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO3 (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO3. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na2SO4 and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).

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Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Pseudouridine, 3-methyluridine or 2,4,6-trimethoxy benzene phosphoramidites were incorporated into ribozymes using solid phase synthesis as described by Wincott et al., 1995 supra. The ribozymes were deprotected using the standard protocol described above with the exception of ribozymes with pseudouridine. Pseudouridine-modified ribozymes were deprotected first by incubation at room temperature, instead of at 55°C, for 24 hours in a mixture of ethanolic ammonia (3:1).

15 Example 27: Synthesis of dihydrouridine phosphoramidites

Referring to Figure 42, dihydrouridine phosphoramidite was synthesized based on the method described in Chaix et al., 1989 Nucleic Acid Res. 17, 7381-7393 with certain improvements:

- i. Uridine (1; 10g, 41mmoles) was dissolved in 200 ml distilled water and to the solution 2g of Rh (10% on alumina) was added. The slurry was brought to 60 psi of hydrogen, and hydrogenation was continued for 16hrs. Reaction was monitored by disappearance of UV absorbing material. All of starting material was converted to dihdrouridine (DHU) and tetrahydrouridine (2:1 based on NMR). Tetrahydrouridine was not removed at this step.
- ii. Dihydrouridine (2; 10g, 41mmoles) was dissolved in 400ml dry pyridine; dimethylaminopyridine (0.244g,2mmoles), triethylamine (7.93ml, 56mmoles), and dimethoxytritylchloride (16.3g, 48mmoles) were added and stirred under argon overnight. The reaction was quenched with 50ml methanol, extracted with 400ml 5% sodium bicarbonate, and then 400ml brine. The organic phase was dried over sodium sulphate, filtered, and then dried to a foam. 5'-DMT-DHU (3) was purified by silica gel chromatography (dichloromethane with 0.5-5% gradient of methanol; final yield = 9g; 16.4mmoles).

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III. 5'-DMT-DHU (3; 9.0g, 16.4mmoles) was dissolved in 150ml dry THF. Pyridine (4.9ml, 60mmoles) and silver nitrate (3.35g, 19.7mmoles) were added at room temperature and stirred under argon for 10min., then tert.-butyldimethylsilylchloride (tBDMS-Cl; 3.0g, 19.7mmoles) was added and the slurry was stirred under argon overnight. The reaction was filtered over celite into 500ml aqueous 5% sodium bicarbonate and then extracted with 200ml chloroform. The organic phase was washed with 250ml brine, dried over sodium sulfate, and then evaporated to a yellow foam. 2'-tBDMS, 5'-DMT-DHU (5) was purified by silica gel chromatography away from the 3'-tBDMS, 5'-DMT-DHU (4) (hexanes with 10-50% gradient ether; final yield = 5.1g; 7.7mmoles), dried over sodium sulfate, filtered, and then dried to a white powder. The product was kept under high vacuum for 48hrs.

iv. 5'-DMT, 2'-tBDMS-DHU (5; 2.10g, 3.17mmoles) was dissolved in 40ml anhydrous dichloromethane. NN-dimethylaminopyridine (2.21ml, 12.7mmoles), N-methylimidizole (1.27ml, 1.59mmoles), and chloro-diisopropyl-cyanoethylphosphoramidite (1.2ml, 5.22mmoles) were added and the reaction was stirred under arc in for 3hrs. The reaction was quenched with 4ml anhydrous methanol and then apporated to an oil. Final product (6) was purified by silicated to chromatography (dichloromethane with 0-1% ethanol; 1% triethylamine; final yield = 2.2g; 2.5mmoles).

The dihydrouridine was incorporated into ribozymes using solid phase synthesis as described by Wincott et al., 1995 supra. with improvements—nuceloside-oxalyl-polystyrene derivatized support (Alul et. al. Nucleic Acids Res., 1991, 19, 1527-1532) was used. The ribozyme containing the dihydrouridine substitution was deprotected using 30% methyl amine in anhydrous ethanol for 15 min. at room temperature and subsequent treatment with tert-butyl-ammonium fluoride in anhydrous THF for 24 hrs. at room temperature.

Example 28: Synthesis of 2-O-f-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-naphthyl-β-D-ribofuranose (7) phosphoramidites

1-Deoxy-1-naphthyl-B-D-ribofuranose (4)

Referring to Figure 45, the title compound was synthesized from naphthalene 1 and tetra-O-acetyl-β-D-ribofuranose 2 according to the procedure of Ohrui et al. Agr. Biol. Chem. 1972, 36, 1651-1653.

2-*O-t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl-β-D-ribofuranose (7)

7 was synthesized in three steps from 4: a) 5'-O-dimethoxytritylation using 4,4'-dimethoxytrityl triflate, followed by chromatographic separation of α and β anomer, respectively; b) 2'-O-silylation was carried out as described by Hakimelahi *et al.*, 1982 *supra* (32% yield); c) 3'-O-phosphitylation was carried out essentially as described by Tuschl *et al.*, 1993 *supra* (85% yield).

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott et al., 1995 supra. The ribozyme containing naphthyl substitution was deprotected using the standard protocol described above.

Example 29: Synthesis of 2-*O*-t-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cy: noethyl-N.N-diisopropylphosphoramidite)-1-Deoxy-1-(p-Aminophenyl)-β-D-Ribofuranose phosphoramidites

5-O-t-Butyldiphenylsilyl-2.3-O-isopropylidene-1-deoxy-1-(p-bromophenyl)-β-D-ribofuranose (3)

20 Referring to Figure 46, 3 was prepared from 4-bromo-1-lithiobenzene and t-butyldiphenylsilyl-2,3-O-isopropylidene-D-ribono-1,4-lactone using the procedure analogous to that described by Czernecki and Ville, J. Org. Chem. 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using instead of t-butyldimethylsilyl the more acid resistant t-butyldiphenylsilyl group for 5-O-protection.

5-O-t-Butyldiphenylsilyl-2.3-O-isopropylidene-1-deoxy-1-(p-aminophenyl)-β-D-ribofuranose (5)

Compound 3 was aminated using liquid ammonia and Cul as described by Piccirilli et al. Helv. Chim. Acta 1991, 74, 397-406 to give the title compound in 63% yield.

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<u>5-O-t-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-[p-(N-TFA) aminophenyl]-β-D-ribofuranose (6)</u>

5 (1.2 g, 2.88 mmol) in dry pyridine (20 ml) was treated with trifluoroacetic anhydride (0.5 ml, 3.6 mmol) for 1 hour at 0 °C. The reaction mixture was then quenched with methanol (5 ml) and evaporated to a syrup. The syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was dried (Na₂SO₄) and evaporated to dryness under reduced pressure. This material was used without further purification in the next step.

1-Deoxy-1-[p-(N-TFA)aminophenyl]-β-D-ribofuranose (7)

The title compound was prepared from 6 in an identical manner as for the synthesis of deblocked phenyl analog; (82% overall yield for 5'-O-desilylation and the cleavage of 2',3'-O-isopropylidene group).

2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-[p-(N-TFA) aminophenyl]-β-D-ribofuranose (10)

Using the same three ; sequence as for the phenyl analog, 10 was prepared from 7 in 32% overall yield.

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott et al., 1995 supra. The ribozyme containing aminophenyl substitution was deprotected using the standard protocol described above.

Example 30: RNA cleavage reactions catalyzed by HH-B substituted with modified bases

Hammerhead ribozymes targeted to site B (see Fig. 43A) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were substituted with various base-modifications shown in Figure 43B.

RNA cleavage reactions were carried out as described above. Referring to Fig. 43B, hammerhead ribozymes containing base modifications at positions 4 or 7 cleave the target RNA to varying degrees of efficiency. Some of the base modifications at position 7 appear to enhance the catalytic efficiency of the

hammerhead ribozymes compared to a standard base at that position (see Figure 43B, pyridin-4-one, phenyl and 3-methyl U modifications).

HH-B ribozymes with either pyridin-4-one or phenyl substitution at position 7 were further characterized (Figure 44). It appears that HH-B ribozyme with pyridin-4-one modification at position 7 cleaves RNA with a 10 fold higher k_{cat} when compared to a ribozyme with a U at position 7 (compare Figure 44 A with 44 B). HH-B ribozyme with a phenyl group at position 7 cleaves RNA with a 3 fold higher kcat when compared to a hammerhead ribozyme with U at position 7 (see Figure 44C).

Sequences listed in Figure 23, 31, 33, 35, 43 and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 31: 2'deoxy-2'-alkylnucleotides

Table D2 is a summary of specified catalytic parameters (t_A and t_S) on short substrates *in vitro*, and stabilities of the noted modified catalytic nucleic acids in human serum. U4 and U7 refer to the uracil bases noted in Figure 1. Modifications at the 2'-position are shown in the table.

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Entry	Modification	t _{1/2} (m) Activity (t _A)	t _{1/2} (m) Stability (ts)	$\beta = t_S/t_A$ x 10		
1	.U4 & U7 = U	1	0.1	1		
2	U4 & U7 = 2'- <i>O</i> -Me-U	4	260	¹ 650		
3	$U4 = 2' = CH_2 - U$	6.5	120	180		
4	$U7 = 2' = CH_2 - U$	8	280	350		
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130		
	114 01 05 11					
6	U4 = 2'=CF ₂ -U	5	320	640		
7 .	U7 = 2'=CF ₂ -U	4	220	550		
8	U4 & U7 = 2'=CF ₂ -U	20	320	160		
9	U4 = 2'-F-U	4	320	800		
10	U7 = 2'-F-U	8	400	500		
11	U4 & U7 = 2'-F-U	4	300	750		
12	U4 = 2'-C-Allyl-U	3	>500	>1700		
13	U7 = 2'-C-Allyl-U	3	220	730		
14	U4 & U7 = 2'-C-AllyI-U	3	120	400		
15	U4 = 2'-araF-U	5	>500	>1000		
16	U7 = 2'-araF-U	4	350	875		
17	U4 & U7 = 2'-araF-U	15	500	330		
18	$U4 = 2'-NH_2-U$	10	500	500		
19	$U7 = 2'-NH_2-U$	5	500	1000		
20	$U4 \& U7 = 2'-NH_2-U$	2	300	1500		
0.4						
21	U4 = dU	6	100	170		
22	U4 & U7 = dU	4	240	600		

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Figure 47 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. Referring to Figure 47, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 48 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang et al. Biochemistry 1992, 31, 5005-5009 and Paolella et al. EMBO J. 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 47 and Table D2 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table D2, all 2'-modified ribozymes showed very large and significant increas: in stability in human serum (shown) and in the other fluids described below (Example 3, data not shown). The order of most aggressive nuclease activity was fetal bovine serum > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table D2). This β value indicated that all modified ribozymes tested had significant, >100 ->1700 fold, increases in overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 48 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 53 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are other non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance. These examples are diagrammed in Figs 48-54.

Example 32: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkylnucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cederaren.R.J. J. Am. Chem. Soc. 1987, 109, 7845-7854 and in 10 Scaringe, S.A.; Franklyn, C.; Usman, N. Nucleic Acids Res. 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein et al. 15 International Publication No. WO 92/07065; and 5 Kois et al. Nucleosides & Nucleotides 1993, 12, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 5. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead 20 ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 33: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were – 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis.

Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 34: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 5 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 10 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in 15 the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from that graph.

Example 35: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to Nucleic Acid Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

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Example 36: 3'.5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 37: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

10 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, 15 and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated, 20 dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 38: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N.N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated in vacuo (40 °C)

and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78~g (90%), white foam.

Example 39: 3'.5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N4-Acetyl-5 Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added 10 dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated in vacuo to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The resulting foam was 15 dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was 20 quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as 25 a white foam.

Example 40: 5'-O-Dimethoxytrityl-2'-C-Allyl-N4-Acetyl-Cytidine

This compound was obtained analogously to the undine derivative 9 in 55% yield.

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Example 41: 5'-O-Dimethoxytrityl-2'-C-allyl-N4-Acetyl-Cytidine 3'-(2-Cyanoethyl N.N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated in vacuo (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 42: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine
14 (Hansske,F.; Madej,D.; Robins,M. J. Tetrahedron 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. J. Med. Chem. 19"1, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with i M TBAF in THF (10 mL) for 20 m and concentrated in vacuo. The residue was triturated with petroleum ether and chromatographed on a silica gel column.
20 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 43: 5'-O-DMT-2'-Deoxy-2'-Methylene-Undine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

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Example 44: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 45: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisilox-ane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A v · ··· (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated in vacuo. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 46: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

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Example 47: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16) :

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

Example 48: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N.N-diisopropylphosphoramidite) (18)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R₁ 0.48 (CH₂Cl₂: MeOH / 15:1).

Example 49: 2'-Deoxy-2'-Methylene-3'.5'-O-(Tetraisopropyldisiloxane-1.3-diyl)-4-N-Acetyl-Cytidine 20

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq.

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ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

10 <u>Example 50: 1-(2'-Deoxy-2'-Methylene-5'-*O*-Dimethoxytrityl-β-D-ribofurano-syl)-4-*N*-Acetyl-Cytosine 21</u>

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

Example 51: 1-(2'-Deoxy-2'-Methylene-5'-*O*-Dimethoxytrityl-β-D-ribofurano-syl)-4-*N*-Acetyl-Cytosine 3'-(2-Cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (22)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture

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was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup in vacuo (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. Rf 0.36 (CH₂Cl₂:MeOH / 20:1).

Example 52: 2'-Deoxy-2'-Difluoromethylene-3'.5'-O-(Tetraisopropyl disiloxane-1.3-divl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine 23 ([described in example 45] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated in vacuo, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated in vacuo; dissolved in dioxane (20 mL) and ag. ammonia (30 mL). The mixture was stirred for 12 h and concentrated in vacuo. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO3 (5mL). The mixture was concentrated in vacuo, dissolved in CH2Cl2 (2 x 100 mL) and washed with 5% NaHCO3 (1 x 100 mL). The organic extracts were dried over Na2SO4. concentrated in vacuo and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-4-Nacetyl-cytidine 24 (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 53: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-

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4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-CI (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

Example 54: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-10 ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-N.N-diisopropylphosphoramidite) (26)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-4-N-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N, N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 55: 2'-Keto-3'.5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butyl-benzoyl)-Adenosine (28)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraiso-propyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (Brown,J.; Christodolou, C.; Jones,S.; Modak,A.; Reese,C.; Sibanda,S.; Ubasawa A. J. Chem .Soc. Perkin Trans. I 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated in vacuo. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated in vacuo. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

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Example 56: 2'-Deoxy-2'-methylene-3'.5'-O-(Tetraisopropyldisiloxane-1,3-divi)-6-N-(4-t-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g,17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCI. The organic layer was washed with H2O (20 mL), 5% aqueous NaHCO3 (20 mL), H2O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated in vacuo to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum. ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).

Example 57: 2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated in vacuo. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

Example 58: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine (29)

2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-CI (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in

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CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).

5 Example 59: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N.N-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-t-butylbenzoyl)-adenine **29** dissolved in dry CH₂Cl₂ (15 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

Example 60: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisilox-ane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of -1 h. The resulting mixture was further stirred for 2 h and concentrated in vacuo. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 61: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) 6-N-(4-t-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL)

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was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated in vacuo. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH₂Cl₂.

Example 62: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated in vacuo and the residue taken up in CH2Cl2 (100 mL) and washed with sat. NaHCO3, water and brine. The organic extracts were dried over MgSO4, concentrated in vacuo and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 63: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-6-N-(4-t-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine 20 (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2cyanoethyl N,N-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup in vacuo (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. Rf 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 64: 2'-Deoxy-2'-Methoxycarbonylmethylidine-3'.5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine (33)

30 Methyl(triphenylphosphoranylidine)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-O-(tetraisopropyl disiloxane-1.3-diyl)-uridine 14 in

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CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 65: 2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (34)

Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxy-carboxylmethylidine-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 33 (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated in vacuo after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidine-uridine 34 (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

<u>Example 66: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine</u> (35)

2'-Deoxy-2'-methoxycarbonylmethylidine-uridine 34 (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 35 (2.03 g, 3.46 mmol, 86%).

Example 67: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidine-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine 35 (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL,

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6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup in vacuo (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 36 (1.8 g. 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. Rf 0.44 (CH₂Cl₂:MeOH/ 9.5:0.5).

Example 68: 2'-Deoxy-2'-Carboxymethylidine-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropyldisilox-ane-1,3-diyl)-uridine 33 (5.0 g. 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidine-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 37 (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

20 <u>Example 69: Synthesis of 2'-C-allyl-U phosphoramidite from 5'-O-DMT-3'-O-TBDMS-Uridine</u>,

Referring to Figure 54, in order to simplify the synthetic scheme for phosphoramidites 5 and 8 we also explored the potential of 5'-O-DMT-3'-O-TBDMS-Uridine 10 (side product in preparation of standard RNA monomers) as a starting material in the synthesis of key intermediate 4. Phenoxythiocarbonylation of starting synthon 10 according to Robins (Robins, M. J., Wilson J. S. and Hansske, F. (1983), *J. Am. Chem. Soc.*; 105, 4059) surprisingly led to thioester 11 (91 %) without noticeable migration (Scaringe, S.A., Franclyn, C. & Usman, N. (1990) *Nucleic Acids Res.*, 18, 5433-5441) of the TBDMS group. Comparative analysis of ¹H NMR data for compounds 10 and 11 revealed that resonance of H-2' experienced up field shift of 2,0 ppm(from 6,06 to 4,13) in 11 compare to starting compound 10, at the same time chemical shift of H-3' and H-1' changed only slightly: 4.83 ppm(H-3') and

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6.48 ppm (H-1') in 11 compare to 4.36(H-3') ppm and 5.93 ppm (H-1') in 10 and chemical shift of H-4' remains practically unchanged indicating acylation at C-2-OH. Heck allylation of intermediate 11 with 2-,2'-Azobis-(2-methyl propionitrile) (other groups can be introduced by standard procedures) resulted in a formation of 2'-C-allyl derivative 12 (70 %) and related 2'-deoxy by-product (15%). Subsequent desilylation of 12 led to 5'-C-DMT derivative 4 identical to the one synthesized from thioester 2. Since the starting material for this route is commercially available this may represent a less laborious way to key synthon 4 as well as for other 2'- modified monomers. This methodology can be used to introduce other 2'-C-allyl groups using compound 11 (or its equivalent for other bases) as an intermediate.

Example 70: Synthesis of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-bytuldimethylsilyl-uridine 11.

To a stirred solution of 5'-O-Dimethoxytrityl-3'-O-t-bytuldimethylsilyl-uridine (Commercially available from Chem Genes Corporation) (5,0 g 7,57 mmol) and dimethylaminopyridine (1,8g, 15 mmol) in 100 ml of dry acetonitril a solution of phenylchlorothionoformate (1.26ml, 9,1 mmol) in 25 ml of acetonitrile was added dropwise and the reaction mixture stirred at room temperature for 3 hours. TLC (ethylacetate-hexanes 1:1) showed disappearance of starting material and the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography on silica gel CH₂Cl₂ as an eluent to give 5.51g (91.3%) of the product.

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃) 3.57 (2H, H5', H5", m J5',4'=2.4., J5",4'=2.8., J5',5'=11.0), 3.86 (6H, OCH₃, s), 4.07 (1H, H4', m), 4.83 (1H, H3', dd, J3',4'=2,8 J3',2'=5,2), 5.44 (1H, H5, d, J5,6=8.0) 5.99 (1H, H2', dd, J2',1'=6.4 , J2',3"= 5,2), 6.46 (1H, H1', d, J1',2'=6.4) , 6.89-7.79 (18H, DMT, Phe, m), 7.88 (1H, H6, d, J₆,5=8.0), 7.95 (1H, N-H, bs).

Example 71: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-bytuldimethylsilyl-uridine(12)

To a refluxing under argon solution of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-bytuldimethylsilyl-uridine (5,5g, 6,9 mmol) and

allyltributyltin (10,7ml, 34,5 mmol) in dry toluene (150 ml) a solution of 2-,2'-Azobis-(2-methyl propionitrile) (0.28g 1,72 mmol) in 50 ml of dry toluene was added dropwise for 1 hour. The resulting mixture was allowed to reflux under argon for additional 2 hours. After that it was concentrated in vacuo and purified by flash chromatography on silica gel with gradient ethylacetate in hexanes (0-30%) as an eluent. Yield 3.38g (70.0%).

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃),2.23 (1H, H6', m), 2.38-2.52 (2H, H6" and H2', m), 3.46 (2H, H5' and H5", m, J $_5$ ',4'=2.5., J $_5$ '',4'=3.2 J $_5$ ',5''=10.8), 3.86 (6H, OCH₃, s), 4.13 (1H, H4', dd, J $_4$ ',3'=8.0, J $_4$ ',5'=3.2,J $_4$ ',5'=2.5), 4.46 (1H, H3', m), 5.15 (1H, H8', d, J $_8$ ',7'=10.0), 5.20 (1H, H9', d, J $_9$ ',7'=17.3), 5.44 (1H, H5, d, J $_5$,6=8.0), 5.81 (1H, H7', dddd, J $_7$ ',6'=6.0, J $_7$ ',6"=8.0), 6.14 (1H, H1', d, J $_1$ ',2'=8.0), 6.88-7.52 (13H, DMT, m), 7.76 (1H, H6, d, J $_6$,5=8.0), 8.17 (1H, N-H, bs)

Example 72: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl Undine (4) from 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-bytuldimethyl-silyl-uridine (12).

Standard deprotection of TBDMS derivative 12 utilizing general method A furnis: .d product 4 (yield 80%) identical to the compound prepared from 2'-C-allyl derivative 3.

<u>Uses</u>

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The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al. PCT WO 94/02595.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-O-methylthioalkyl-substituted phosphoramidites and the syntheses of the amidites.

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Example 73: Synthesis of Hammerhead Ribozymes Containing 2'-Oalkylthioalkylnucleotides & Other Modified Nucleotides

The method of synthesis follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. J. Am. Chem. Soc. 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. Nucleic Acids Res. 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. These 2'-Ó-alkylthioalkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 74: Synthesis of base-protected 3'.5'-O-(tetraisopropyldisiloxane-1.3-divl) nucleosides (2)

Referring to Figure 55, standard introduction of "Markiewicz" protecting 15 group to the base-protected nucleosides according to "Oligonucleotides and Analogues. A Practical Approach", ed. F. Eckstein, IRL Press, 1991 resulted in protected nucleosides (2) with 85-100% yields. Briefly, in a non-limiting example, Uridine (20g, 81.9 mmol) was dried by two coevaporations with anhydrous pyridine and re dissolved in the anhydrous pyridine. The above 20 solution was cooled (0°C) and solution of 1,3-dichloro-1,1,3,3tetraisopropylsiloxane (28.82 mL, 90.09 mmol) in 30 mL of anhydrous dichloroethane was added dropwise under stirring. After the addition was completed the reaction mixture was allowed to warm to room temperature and stirred for additional two hours. Then it was quenched with MeOH (25 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated NaHCO3 and brine. The organic layer was evaporated to dryness and then coevaporated with toluene to remove traces of pyridine to give 39g (98%) of compound 2 (B=Ura) which was used without further purification.

Other 3',5'-O-(tetraisopropyldisiloxane-1,3-di-yl)- nucleosides were obtained in 75-90% yields, using the protocol described above, starting from

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base-protected nucleosides with final purification of the products by flash chromatography on silica gel when necessary.

Example 75: General procedure for the synthesis of 2'-O-methylthiomethyl nucleosides (3)

Referring to Figure 55, to a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) nucleoside (2) (7 mmol), methyl disulfide (70 mmol), 2,6-lutidine (7 mmol) in methylene chloride (100 mL) or mixture methylene chloride - acetonitrile (1:1) under positive pressure of argon, solution of benzoyl peroxide (28 mmol) in methylene chloride was added dropwise during 1 hour. After complete addition the reaction mixture was stirred at 0°C under argon for additional 1 hour. The solution was allowed to warm to room temperature, diluted with methylene chloride (100 mL), washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using 1-2% methanol in methylene chloride as an eluent to give corresponding methylthiomethyl nucleosides with 55: % yield.

Example 76: 5'-O-Dimethoxytrityl-2'O-Methylthiomethyl-Nucleosides. (6)

Method A. The solution of the base-protected 3',5'-O- (tetraisopropyldisiloxane-1,3-diyl)-2'-O-methylthiomethyl nucleoside (3) (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. Resulting mixture was evaporated, the residue was loaded to the silica gel column, washed with 1L of chloroform, and the desired deprotected compound was eluted with 5-10% methanol in dichliromethane. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 ml), evaporated, dissolved in chloroform, washed with saturated aq sodium bicarbonate and brine. Organic layer was dried over sodium sulfate and evaporated. The residue was purified

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by flash chromatography on silica gel to give 5'-O-Dimethoxytrityl derivatives with 70-80% yield.

Method B. Alternatively, 5'-O-Dimethoxytrityl-2'O-Methylthiomethyl-Nucleosides (6) may also be synthesized using 5'-O-Dimethoxytrityl-3'-O-t-Butyl-dimethy-Isilyl Nucleosides (4) as the starting material. Compound 4 is commercially available as a by-product during RNA phosphoramidite synthesis. Compond 4 is converted in to 3'-O-t-butyldimethylsilyl-2'-O-methylthiomethyl nucleoside 5, as described under example 3. The solution of the base-protected 3'-O-t-butyldimethylsilyl-2'-O-methylthiomethyl nucleoside 5 (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. The resulting mixture was evaporated, and purified by flash silica gel chromatography to give nucleosides 6 in 90% yield.

Example 77: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides-3'-(2-Cyanoethyl-N,N-diisopropylphosphoroamidites) (7)

Standard phosphitylation of nucleoside 6 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 78: General procedure for the synthesis of 2'-O-Methylthiophenyl nucleosides.

To a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) nucleoside (14,7 mmol), thioanisole (147 mmol), N,N-dimethylaminopyridine (58.8 mmol) in acetonitrle (100 mL) under positive pressure of argon, benzoyl peroxide (36.75 mmol) was added portionwise over 3 hours. After complete addition the reaction mixture was allowed to warm to room temperature and was stirred under argon for an additional 1 hour. The solvents were removed in vacuo, the residue was dissolved in ethylacetate, washed twice with saturated and NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture EtOAc-hexanes (1:1) as eluent to give the corresponding methylthiophenyl nucleosides with 55-65% yield.

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Example 79: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides.

These compounds were prepared as described above under examples 76 and 76.

Example 80: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides-3'-(2-Cyanoethyl N.N-diisopropylphosphoroamidites)

Standard phosphitylation according to Scaringe, S.A.; Franklyn, C.; Usman, N. Nucleic Acids Res. 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 81: Ribozymes containing 2'-O-methylthiomethyl substitutions

In a non-limiting example 2'-O-methylthioalkyl substitutions were made at various positions within a hammerhead ribozyme motif (Fig. 56, including U4 and U7 positions). The target site B was targeted by the hammerhead ribozyme in this non-limiting example.

Hammerhead ribozymes (see Fig. 56) were synthesized using solidphase synthesis, as describe above. Several positions were modified, individually or in combination, with 2'-O-methylthiomethyl groups.

RNA cleavage assay in vitro:

Substrate RNA is 5' end-labeled using [γ -32P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (\leq 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

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Referring to Figure 57, hammerhead ribozymes containing 2'-O-methylthiomethyl modifications at various positions cleave the target RNA efficiently. Surprisingly, all the 2'-O-methylthiomethyl -substituted ribozymes cleaved the target RNA more efficiently compared to the control hammerhead ribozyme.

Sequences listed in Figure 56 and the modifications described in Figure 56 and 57 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other combinations of 2'-hydroxyl group modifications can be readily generated using techniques known in the art, and are within the scope of the present invention.

The following are non-limiting examples showing the synthesis of non-nucleotide mimetic-containing catalytic nucleic acids using non-nucleotide phosphoramidites.

Such non-nucleotides can be located in the binding arms, core or the loop adjacer, stem II of a hammerhead type ribozyme. Those in the art following the teachings herein can determine optimal locations in these regions. Surprisingly, abasic moieties can be located in the core of such a ribozyme.

Example 82: Synthesis of Abasic nucleotides

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite 9 is shown in Figure 58. Our initial efforts concentrated on the deoxygenation of synthon 1, prepared by a "one pot" procedure from D-ribose. Phenoxythiocarbonylation of acetonide 1 under Robins conditions led to the β-anomer 2 (J_{1,2} = 1.2 Hz) in modest yield (45-55%). Radical deoxygenation using Bu₃SnH/AIBN resulted in the formation of the ribitol derivative 3 in 50% yield. Subsequent deprotection with 90% CF₃COOH (10 m) and introduction of a dimethoxytrityl group led to the key intermediate 4 in 40% yield (Yang et al., *Biochemistry* 1992, 31, 5005-5009; Perreault et al., *Biochemistry* 1991, 30, 4020-4025; Paolella et al., *EMBO J.* 1992, 11, 1913-1919; Peiken et al., *Science* 1991, 253, 314-317).

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The low overall yield of this route prompted us to investigate a different approach to 4 (Fig. 58). Phenylthioglycosides, successfully employed in the Keck reaction, appeared to be an alternative. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on Bu₃SnH concentration). Therefore we subjected phenylthioglycoside 5 to radical reduction with Bu₃SnH (6.1 eq.) in the presence of Bz₂O₂ (2 eq.) resulting in the isolation of tribenzoate 6 in 63% yield (Fig. 9B). Subsequent debenzoylation and dimethoxytritylation led to synthon 4 in 70% yield. Introduction of the TBDMS group, using standard conditions, resulted in the formation of a 4:1 ratio of 2- and 3-isomers 8 and 7. The two regioisomers were separated by silica gel chromatography. The 2-O-t-butyldimethylsilyl derivative 8 was phosphitylated to provide phosphoramidite 9 in 82% yield.

15 Example 83: RNA cleavage assay in vitro

Ribozymes and substrate RNAs were synthesized as described above. Substrate RNA was 5' end-labeled using [... P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme were denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate were incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction was initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction quenched by mixing with an equal volume of 2X formamide stop mix. The samples were resolved on 20 % denaturing polyacrylamide gels. The results were quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Figure 59 there is shown the general structure of a hammerhead ribozyme targeted against site B (HH-B) with various bases numbered. Various substitutions were made at several of the nucleotide positions in HH-B. Specifically referring to Figure 60, substitutions were made

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at the U4 and U7 positions marked as X4 and X7 and also in loop II in the positions marked by an X. The RNA cleavage activity of these substituted ribozymes is shown in the following figures. Specifically, Figure 61 shows cleavage by an abasic substituted U4 and an abasic substituted U7. As will be noted, abasic substitution at U4 or U7 does not significantly affect cleavage activity. In addition, inclusion of all abasic moieties in stem II loop does not significantly reduce enzymatic activity as shown in Figure 62. Further, inclusion of a 3' inverted deoxyribose does not inactivate the RNA cleavage activity as shown in Figure 63.

10 Example 84: Smooth Muscle Cell Proliferation Assay

Hammerhead ribozyme (HH-A) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of c-myb protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown et al., 1992 *J. Biol. Chem.* 267, 4625).

The ribozymes that cleaved site A within c-myb RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells we: isolated and cultured as described (Stnchcomb et al., supra). These primary rat aortic smooth muscle cells (RASMC) were plated in a 24-well plate (5x10³ cells/well) and incubated at 37°C in the presence of Dulbecco's Minimal Essential Media (DMEM) and 10% serum for -16 hours.

These cells were serum-starved for 48-72 hours in DMEM (containing 0.5% serum) at 37°C. Following serum-starvation, the cells were treated with lipofectamine (LFA)-complexed ribozymes (100 nM ribozyme was complexed with LFA such that LFA:ribozyme charge ration is 4:1).

Ribozyme:LFA complex was incubated with serum-starved RASMC cells for four hours at 37°C. Following the removal of ribozyme:LFA complex from cells (after 4 hours), 10% serum was added to stimulate smooth cell proliferation. Bromo-deoxyuridine (BrdU) was added to stain the cells. The cells were stimulated with serum for 24 hours at 37°C.

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Following serum-stimulation, RASMC cells were quenched with hydrogen peroxide (0.3% H_2O_2 in methanol) for 30 min at 4°C. The cells were then denatured with 0.5 ml 2N HCl for 20 min at room temperature. Horse serum (0.5 ml) was used to block the cells at 4°C for 30 min up to ~16 hours.

The RASMC cells were stained first by treating the cells with anti-BrdU (primary) antibody at room temperature for 60 min. The cells were washed with phosphate-buffered saline (PBS) and stained with biotinylated affinity-purified anti-mouse IgM (Pierce, USA) secondary antibody. The cells were counterstained using avidin-biotinylated enzyme complex (ABC) kit (Pierce, USA).

The ratio of proliferating:non-proliferating cells was determined by counting stained cells under a microscope. Proliferating RASMCs will incorporate BrdU and will stain brown. Non-proliferating cells do not incorporate BrdU and will stain purple.

Referring to Figure 64 there is shown a ribozyme vinich cleaves the site A referred to as HH-A. Substitutions of abasic moieties i.. place of U4 as shown in Figure 65 provided active ribozyme as shown in Figure 66 using the above-noted rat aortic smooth muscle cell proliferation assay.

The method of this invention generally features HPLC purification of 20 ribozymes. An example of such purification is provided below in which a synthetic ribozyme produced on a solid phase is blocked. This material is then released from the solid phase by a treatment with methanolic ammonia, subsequently treated with tetrabutylammonium fluoride, and purified on reverse phase HPLC to remove partially blocked ribozyme from "failure" 25 sequences. Such "failure" sequences are RNA molecules which have a nucleotide base sequence shorter to that of the desired enzymatic RNA molecule by one or more of the desired bases in a random manner, and possess free terminal 5'-hydroxyl group. This terminal 5'-hydroxyl in a 30 ribozyme with the correct sequence is still blocked by lipophilic dimethoxytrityl group. After such partially blocked enzymatic RNA is purified, it is deblocked by a standard procedure, and passed over the same or a similar HPLC

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reverse phase column to remove other contaminating components, such as other RNA molecules or nucleotides or other molecules produced in the deblocking and synthetic procedures. The resulting molecule is the native enzymatically active ribozyme in a highly purified form.

Below are provided examples of such a method. These examples can be readily scaled up to allow production and purification of gram or even kilogram quantities of ribozymes.

Example 85: HPLC Purification, Reverse-Phase

In this example solid phase phosphoramidite chemistry was employed for synthesis of a ribozyme. Monomers used were 2'-t-butyl-dimethylsilyl cyanoethylphosphoramidites of undine, N-benzoyl-cytosine, N-phenoxyacetyl adenosine, and guanosine (Glen Research, Sterling, VA).

Solid phase synthesis was carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only exception was that the coupling step was increased from 10 to 12 minutes. The phosphorami-lite concentration was 0.1 M. Synthesis was done on a 1 μ mol scale using a 1 μ mol RNA reaction column (Glen Research). The average coupling efficiencies were between 97% and 98% for the 394 model and between 97% and 99% for the 380B model, as determined by a calorimetric measurement of the released trityl cation. The final 5'-DMT group was not removed.

After synthesis, the ribozymes were cleaved from the CPG support, and the base and phosphotriester moieties were deprotected in a sterile vial by incubation in dry ethanolic ammonia (2 mL) at 55 °C for 16 hours. The reaction mixture was cooled on dry ice. Later, the cold liquid was transferred into a sterile screw cap vial and lyophilized.

To remove the 2'-t-butyldimethylsilyl groups from the ribozyme the obtained residue was suspended in 1 M tetra-n-butylammonium fluoride in dry THF (TBAF), using a 20-fold excess of the reagent for every silyl group, for 16 hours at ambient temperature. The reaction was quenched by adding an

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equal volume of a sterile 1 M triethylamine acetate, pH 6.5. The sample was cooled and concentrated on a SpeedVac to half of the initial volume.

The ribozymes were purified in two steps by HPLC on a C4 300 Å 5 μm DeltaPak column in an acetonitrile gradient.

The first step, or "trityl on" step, was a separation of 5'-DMT-protected ribozyme(s) from failure sequences lacking a 5'-DMT group. Solvents used for this step were: A (0.1 M triethylammonium acetate, pH 6.8) and B (acetonitrile). The elution profile was: 20% B for 10 minutes, followed by a linear gradient of 20% B to 50% B over 50 minutes, 50% B for 10 minutes, a linear gradient of 50% B to 100% B over 10 minutes, and a linear gradient of 100% B to 0% B over 10 minutes.

The second step was a purification of a completely deprotected, *i.e.* following the removal of the 5'-DMT group, ribozyme by a treatment with 2% trifluoroacetic acid or 80% acetic acid on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient. Solvents used for this second step were: A (0.1 M Triethylammonium acetate, pH 6.8) and B (80% acetonitrile, 0.1 M triethylammonium acetate, pH 6.8). The elution profile was: ...% B for 5 minutes, a linear gradient of 5% B to 15% B over 60 minutes, 15% B for 10 minutes, and a linear gradient of 15% B to 0% B over 10 minutes.

The fraction containing ribozyme, which is in the triethylammonium salt form, was cooled and lyophilized on a SpeedVac. Solid residue was dissolved in a minimal amount of ethanol and ribozyme in sodium salt form was precipitated by addition of sodium perchlorate in acetone. (K+ or Mg²+ salts can be produced in an equivalent manner.) The ribozyme was collected by centrifugation, washed three times with acetone, and lyophilized.

Example 86: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using ethylamine (EA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of ethylamine (EA) @ 25-55 °C for 10-30 min to remove the exocyclic amino protecting groups (see Figure 67). The supernatant was removed from the polymer support. The support was washed with 1.0 mL of

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EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

Table EVII is a summary of the results obtained using the improvements outlined in this application for base deprotection. From this data it is evident EA at 55° for 10 m or 40° for 10 m is efficient. The HPLC peak structure is almost identical between these schemes, and the yield for the ethylamine deprotected oligos is actually slightly better than the methylamine.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman et al. J. Am. Chem. Soc. 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in N-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results.

The following are examples of preferred embodiments of the present invention. Those in the art will recognize that these are not limiting examples but rather are provided to guide those in the art to the full breadth of meaning of the present invention. Routine procedures can be used to utilize other coupling regions not exemplified below.

Ribozymes were synthesized in two parts and tested without ligation for catalytic activity. Referring to Fig. 72, the cleavage activity of the half ribozymes containing between 5 and 8 base pairs stem IIs at 40 nM under single turnover conditions was comparable to that of the full length oligomer as shown in Figs. 73 and 74. The same half ribozymes were synthesized with suitable modifications at the nascent stem II loop to allow for crosslinking. The halves were purified and chemically ligated, using a variety of crosslinking methods. The resulting full length ribozymes (see Fig. 71) exhibited similar cleavage activity as the linearly synthesized full length oligomer as shown in Fig. 74.

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Example 87

Referring to Fig. 70 the 5' half of a hammerhead ribozyme was provided with a ribose group. This was oxidatively cleaved with NalO₄ and reacted with the 3' half of the ribozyme having an amino group under reducing conditions. The resulting ribozyme consisted of the two half ribozyme linked by a morpholino group.

One equivalent of (200 micrograms) of 5' half hammerhead with a 3'OH and 5 equivalents (1000 micrograms) of 3' half with 5' C5-NH₂ all with HH-A were used in this reaction. The limiting oligonucleotide was oxidized first with 3.6 equivalents of sodium periodate for sixty minutes on ice in DEPC water quenched with 7.2 equivalents of ethylene glycol for 30 minutes on ice and the 5 equivalents of the amino oligo added. 0.5 Molar tricine buffer, pH 9, was added to provide 25 millimolar final tricine concentration and left for 30 minutes on ice. 50 equivalents of sodium cyanoborohydride was then added and the pH reduced to 6.5 with acetic acid and reaction left for 60 minutes on ice. The resulting full length ribozyme was then purified for further analysis.

Example 88: Amide Bond

Referring again to Fig. 70 and 71, a 5' half of ribozyme was provided with a carboxyl group at its 2' position and was coupled with an amine containing 3' half ribozyme. The provision of a coupling reagent resulted in a full-length ribozyme having an amide bond.

Example 89: Disulfide Bond

Referring to Fig. 70 and 71, 250 micrograms of RPI3881 and 250 micrograms of RPI3636 half ribozyme were separately deprotected with dithiothreitol overnight at 37°C. They were mixed together at 1:1 mole ratio in a 100 mM sodium phosphate buffer at pH 8 and 4M copper sulfate and 0.8 mM 1,10-phenanthroline (final concentrations) was added for two hours at room temperature (20-25°C) and the resulting mixture gel purified. The overall purification yield of full length ribozyme was 30%.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.8 KB region (containing site A) was synthesized by PCR using

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primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed, using T7 RNA polymerase, in a standard transcription buffer in the presence of [α -32P]CTP. The reaction mixture was treated with 15 units of ribonuclease-free DNasel, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (200 nM) and internally labeled 1.8 KB substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris·HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 µl were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Few antiviral dang therapies are available that effectively inhibit established viral infections. Consequently, prophylactic immunization has become the method of choice for protection against viral pathogens. However, effective vaccines for divergent viruses such as those causing the common cold, and HIV, the etiologic agent of AIDS, may not be feasible. Consequently, new antiviral strategies are being developed for combating viral infections.

Gene therapy represents a potential alternative strategy, where antiviral genes are stably transferred into susceptible cells. Such gene therapy approaches have been termed "intracellular immunization" since cells expressing antiviral genes become immune to viral infection (Baltimore, 1988 Nature 335, 395-396). Numerous forms of antiviral genes have been developed, including protein-based antivirals such as transdominant inhibitory proteins (Malim et al., 1993 J. Exp. Med., Bevec et al., 1992 P.N.A.S. (USA) 89, 9870-9874; Bahner et al., 1993 J. Virol. 67, 3199-3207) and viral-activated suicide genes (Ashom et al., 1990 P.N.A.S.(USA) 87, 8889-8893). Although

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effective in tissue culture, protein-based antivirals have the potential to be immunogenic *in vivo*. It is therefore conceivable that treated cells expressing such foreign antiviral proteins will be eradicated by normal immune functions. Alternatives to protein based antivirals are RNA based molecules such as antisense RNAs, decoy RNAs, agonist RNAs, antagonist RNAs, therapeutic editing RNAs and ribozymes. RNA is not immunogenic; therefore, cells expressing such therapeutic RNAs are not susceptible to immune eradication.

Example 90: Design and construction of U6-S35 Chimera

A transcription unit, termed U6-S35, is designed that contains the characteristic intramolecular stem of a S35 motif (see Figure 76). As shown in Figure 77, 78 and 79 a desired RNA (e.g. ribozyme) can be inserted into the indicated region of U6-S35 chimera. This construct is under the control of a type 3 pol III promoter, such as a mammalian U6 small nuclear RNA (snRNA) promoter (see Fig. 75). U6-S35-HHI and U6-S35-HHII are non-limiting examples of the U6-S35 chimera.

As a no if niting example, applicant has constructed a stable, active ribozyme RNA driven from a eukaryotic U6 promoter (Fig. 78). For stability, applicant incorporated a S35 motif as described in Fig. 76 and Fig. 77. A ribozyme sequence is inserted at the top of the stem, such that the ribozyme is separated from the S35 motif by an unstructured spacer sequence (Fig. 77, 78, 79). The spacer sequence can be customized for each desired RNA sequence. U6-S35 chimera is meant to be a non-limiting example and those skilled in the art will recognize that the structure disclosed in the figures 77, 78 and 79 can be driven by any of the known RNA polymerase promoters and are within the scope of this invention. All that is necessary is for the 5' region of a transcript to interact with its 3' region to form a stable intramolecular structure (S35 motif) and that the S35 motif is separated from the desired RNA by a stretch of unstructured spacer sequence. The spacer sequence appears to improve the effectiveness of the desired RNA.

By "unstructured" is meant lack of a secondary and tertiary structure such as lack of any stable base-paired structure within the sequence itself, and preferably with other sequences in the attached RNA.

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By "spacer sequence" is meant any unstructured RNA sequence that separates the S35 domain from the desired RNA. The spacer sequence can be greater than or equal to one nucleotide.

In vitro Catalytic Activity of U6-S35-Ribozyme Chimeras:

U6-S35-HHI ribozyme RNA was synthesized using T7 RNA polymerase. HHI RNA was chemically synthesized using RNA phosphoramidite chemistry as described in Wincott et al., 1995 Nucleic Acids Res. The ribozyme RNAs were gel-purified and the purified ribozyme RNAs were heated to 55°C for 5 min. Target RNA used was ~650 nucleotide long. Internally-32P-labeled target RNA was prepared as described above. The target RNA was preheated to 37°C in 50 mM Tris.HCl, 10 mM MgCl₂ and then mixed at time zero with the ribozyme RNAs (to give 200 nM final concentration of ribozyme). At appropriate times an aliquot was removed and the reaction was stopped by dilution in 95% formamide. Samples were resolved on a denaturing urea-polyacrylamide gel and products were quantitated on a phospholmager[®].

As shown in Figure 80, the U6-S35-HHI ribozyme chimera cleaved its target RNA as efficiently as a chemically synthesized HHI ribozyme. In fact, it appears that the U6-S35-HHI ribozyme chimera may be more efficient than the synthetic ribozyme.

20 Accumulation of U6-S35-ribozyme transcripts

An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells. Cells were transfected ovemight with plasmids encoding the appropriate transcription units (2µg DNA/well of 6 well plate) using calcium phosphate precipitation method (Maniatis et al., 1982 *Molecular Cloning* Cold Spring Harbor Laboratory Press, NY). After the overnight transfection, media was replaced and the cells were incubated an additional 24 hours. Cells were then incubated in media containing 5µg/ml Actinomycin D. At the times indicated, cells were lysed in guanidinium isothiocyanate, and total RNA was purified by phenol/chloroform extraction and isopropanol precipitation as described by Chomczynski and Sacchi, 1987 *Anal. Biochem.*, 162, 156. RNA was analyzed by northen blot analysis and the levels of

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specific RNAs were radioanalyticaly quantitated on a phospholmager[®]. The level of RNA at time zero was set to be 100%.

As shown in Figure 81, the U6-S35-HHII ribozyme shown in Figure 79 is fairly stable in 293 mammalian cells with an approximate half-life of about 2 hours.

Example 91: Design and construction of VA1-S35 Chimera

Refering to Figure 83A, In order to express ribozymes from a VAI promoter, applicant has constructed a transcription unit consisting of a wild type VA1 sequence with two modifications: a "S35-like" motif extends from a loop in the central domain (Figure 82); the 3' terminus is changed such that there is a more complete interaction between the 5' and the 3' region of the transcript (specifically, an "A-C" bulge is changed to an "A-U base pair and the termination sequence is part of the stem of S35 motif).

Accumulation of VA1-S35-ribozyme transcripts

An Actinomycin Descay was used to measure accumulation of the transcript in mammalian cells as described above. As shown in Figure 84, the VA1-S35-chimera, shown in Figure 83A, has approximately 10-fold higher stability in 293 mammalian cells compared to VA1-chimera, shown in Figure 25B that lacks the intramolecular S35 motif.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated U6-S35 or VA1-S35 chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in the Figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of

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stromolysin, B7-1, B7-2, B7-3 and/or CD40 or other RNAs in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with B7-1, B7-2, B7-3 and/or CD40 or other RNA related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and

putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., B7-1, B7-2, B7-3 and/or CD40) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

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TABLE 1

Characteristics of Ribozymes

Group I Introns

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

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Table AII: Human Stromelysin Hammerhead Target Sequence

nt		
Position	Sequence	SEQ. ID. NO.
		**
20	UAGAGCUAAGUAAAGCCAG	ID. NO. 01
126	ACACCAGCAUGAA	ID. NO. 02
147	AGAAALIAUTUAGA	ID. NO. 03
171	ACCUCAAAAAAGAUGUGAAAACAGU	ID. NO. 04
240	AAALGCAGAAGUUC	ID. NO. 05
287	GACACUCUGGAGGUGAUGCGCAAGCCCAGGUGU	ID. NO. 06
327	CUGALIGUUGGUCACUUCAGAAC	ID. NO. 07
357	GCAUCCCGAAGUGGAGGAAAACCCACCUUACAU	ID. NO. 08
402	AUUAUACACCAGAUUUGCCAAAAGAUG	ID. NO. 09
429	CUGUCGALUCUGCUGUCAGA	ID. NO. 10
455	CUGAAAGUCUGGGAAGAGGUGA	ID. NO. 11
513	CUGALIALIAALIGA	ID. NO. 12
592	UGCCUAUGCCCC	ID. NO. 13
624	AUGCCCACUUUGAUGAUGAUGAACAAUGGACA	ID. NO. 14
67 !	ALUUCUCGUUGCUCCUCALIG	ID. NO. 15
725	CACUCAGOCAACACUGA	ID. NO. 16
801	AAGALGALIALIAAAUGGCALUCAGUCC	ID. NO. 17
827	CUCUALOGACCUCCCCCU	ID. NO. 18
859	CCCCCUGGUACOCA	ID. NO. 19
916	UCCUGCUUUGUCCUUUGAUGCUCUCAGCAC	ID. NO. 20
958	AALICCUGAUCUUUAAAGA	ID. NO. 21
975	CAGGCACUUUGGCGCAAAUCCC	ID. NO. 22
1018	ALUGCALUUGAUCUCUCALUUUGGCCAUC	ID. NO. 23
1070	GCALIALIGAAGUUA	ID. NO. 24
1203	AAAUCGAUGCAGCCAUUUCUGA	
1274	UUUGAUGAGAAGAGAAAUUCCAUGGAGC	ID. NO. 26
1302	CAGGCUUUCCCAAGCAAAUAGCUGAAGAC	□ NO. 27
1420	CCCAAAUGCAAAG .	D. NO. 28
1485	AUGUAGAAGGCACAAUAUGGGCACUUUAAA	ID. NO. 29
1623	UCUUGCOGGUCALUUUIIALIGUUALI	D. NO. 30
1665	GOUGOUUAGO	ID. NO. 31

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	140		
1733	CAACAGACAAGUGACUGUAUCU	ID. NO. 32	•
1769	CULALULIAAUA	ID. NO. 33	
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Table AIII: Human Stromelysin HH Target Sequence

nt. Position	Target Sequence S	eq. ID. NO.
10	GCAAGGCALIA GAGACAACALIAGAGC	ID. NO. 34
21	GCAUAGAGACAACAUA GAGCUAAGUAAAGC	ID. NO. 35
27	AGACAACALIAGAGOUA AGUAAAGOUAGUGG	ID. NO. 36
31	AACAUAGAGCUAAGUA AAGCCAGUGGAAAU	E ID. NO. 37
53	GUGGAAAUGAAGAGUC UUCCAAUCCUACUG	J ID. NO. 38
55	GGAAAUGAAGAGUCUU OCAAUOOUACUGUU	ID. NO. 39
56	GAAAUGAAGAGUCUUC CAAUCCUACUGUUG	ID. NO. 40
ਗ	GAAGAGUCUUCCAAUC CUACUGUUGCUGUG	
64	GAGUCUUCCAAUCCUA CUGUUGCUGUGCGU	ID. NO. 42
69	UUCCAAUCCUACUGUU GCUGUGGGUGGCAGU	ID. NO. 43
85	GCUGUGGUGGCAGUU UGCUCAGCCUAUCCA	ID. NO. 44
86	CUC : SUCCOCACUUU CCUCACCCUAUCCAU	ID. NO. 45
90	GCGUGGCAGUUUGCUC AGCCUAUCCAUUGGA	ID. NO. 46
96	CAGUUUGCUCAGCCUA UCCAUUGGAUGGAGC	ID. NO. 47
98	GUUUGCUCAGOCUAUC CAUUGCALOGAGOUG	ID. NO. 48
102	GCUCAGCCUAUCCAUU GGAUGGAGCUGCAAG	ID. NO. 49
142	CACCAGCAUGAACCUU GUUCAGAAAUAUCUA	ID. NO. 50
145	CAGCALIGAACCUUGUU CAGAAALIAUCUAGAA	ID. NO. 51
146	AGCAUGAACCUUGUUC AGAAALIAUCUAGAAA	ID. NO. 52
153	ACCUUGUUCAGAAALIA UCUAGAAAACUACUA	ID., NO. 53
155	CUUGUUCAGAAAUAUC UAGAAAACUACUACG	ID. NO. 54
157	UGUUCAGAAALIAUGUA GAAAAGUACUACGAC	ID. NO. 55
165	AALIAUCUAGAAAACUA CUACGACCUCAAAAA	ID. NO. 56
168	AUCUAGAAAACUACUA CGACCUCAAAAAACA	ID. NO. 57
175	AAACUACUACGACCUC AAAAAAGAUGUGAAA	ID. NO. 58
195	AAGAUGUGAAACAGUU UGUUAGGAGAAAGGA	ID. NO. 59
196	AGAUGUGAAACAGUUU GUUAGGAGAAAGGAC	ID. NO. 60

199	nancyathachennenn yegyeyyygencyen	ID. NO. 61
200	GUGAAACAGUUGUUA GGAGAAAGGACAGUG	ID. NO. 62
218	AGAAAGGACAGUGUUC CUGUUGUAAAAAAA	ID. NO. 63
223	GCACAGUGGUCUGUU GUUAAAAAAAUCOGA	ID. NO. 64
226	CAGUGGUCCUGUUGUU AAAAAAAAUCCCGAGAA	ID. NO. 65
227	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	ID. NO. 66
235	USIKUSUUAAAAAANIC OSAGAAAUSCAGAAG	ID. NO. 67
252	CAGAAAUGCAGAAGUU COUUGGAUUGGAGGU	ID. NO. 68
253	AGAAAUGCAGAAGUUC CUUGGAUUGGAGGUG	ID. NO. 69
256	AAUGCAGAAGUUCCUU GGAUUGGAGGUGACG	ID. NO. 70
261	AGAAGUUCCUUGAUU GGAGGUGACGGGAAA	ID. NO. 71
285	CCCCCAACCUCCACACUCUCCACCOU	ID. NO. 72
293	CUCCACUCCIACACUC UCCACGUEAUCOCCA	ID. NO. 73
325	CCCACCUCICACUU CCUCAUGUCAC	ID. NO. 74
326	COCAGGIGIAGAGIAC CACATIGIAGACACA	ID. NO. 75
334	UGGAGUUCCUGAUGUU GGUCACUUCAGAACC	ID. NO. 76
338	GUCCUGALGUEGUC ACUICAGAACCUUC	ID. NO. 77
342	CREATICATEC CACAVCCATACCATACCATACCATACCATACCATACCA	ID. NO. 78
343	UGALGUUGGUCACUUC AGAACCUUUCCUGGC	ID. NO. 79
351	GUCACUUCAGAACOJU UCCUGGCALICOOGAA	ID. NO. 80
352	UCACUUCAGAACCUUU CCUGGCAUCCCGAAG	ID. NO. 81
353	CACTUCAGAACCUUC CUGGCAUCCGAAGU	ID. NO. 82
361	AACCUUUCCUGGCAUC CCGAAGUGGAGAAA	ID. NO. 83
385	GAGGAAAACCCACCUU ACAUACAGGAUUGUG	ID. NO. 84
386	AGGAAAACOCACCUUA CAUACAGGAUUGUGA	ID. NO. 85
390	AAACCCACCUUACAUA CAGGAUUGUGAAUUA	ID. NO. 86
397	CCULACALIACAGGALU GUGAALUALIACACCA	ID. NO. 87
404	UACAGGAUUGUGAAUU ALIACACCAGAUUUGC	ib. vo. 88
405	ACAGGALUGUGAALUA UACACCAGALUUGCC	ID. NO. 89
407	AGGALIUGUGAALUIALIA CACCAGALUUGCCAA	ID. NO. 90
416	AAUUAUACACCAGAUU UGCCAAAAGAUGCUG	ID. NO. 91
417	AUUAUACACCAGAUUU GCCAAAAGAUGCUGU	
433	COCAAAACAUGCUGUU GAUUCUGUGUGAG	
437	AAAGAUGCUGUGALU CUGCUGUGAGAAAG	
438	AAGAUGCUGUGALUC UGCUGUGAGAAAGC	
445	UGUUGALUCUGUGUU GAGAAACCUCUGAAA	ID. NO. 96

455	GCUGUGAGAAAGCUC UGAAAGUCUGGGAAG	ID. NO. 97
463	GAAAGCUCUGAAAGUC UGGGAAGAGGTUGACU	ID. NO. 98
479	UGGGAAGAGGUGACUC CACUCACAUUCUCCA	ID. NO. 99
484	AGAGGUGACUCCACUC ACAUUCUCCAGGCUG	ID. NO. 100
489	UGACUCCACUCACALU CUCCAGGCUGUAUGA	ID. NO. 101
490	GACUCCACUCACALUC UCCAGGOUGUAUGAA	ID. NO. 102
492	CUCCACUCACAUUCUC. CAGGCUGUAUGAAGG	ID. NO. 103
501	CAUUCUCCAGGCUGUA UGAAGGAGACGCUGA	ID. NO. 104
518	GAAGGAGAGCUGALIA UAAUGALICUOUUUUG	ID. NO. 105
520	AGGAGAGGCUGALIALIA AUGALICUCUUUUGCA	ID. NO. 106
526	GOCUGALIALIAALIGALIC UCUULUGCAGULIAGA	ID. NO. 107
528	CUGALIALIAALIGALICUC UUUUGCAGULIAGAGA	ID. NO. 108
530	CALIALIAAUGAUCUCUU UUGCAGUUAGAGAAC	ID. NO. 109
531	ALIALIAAUGAUCUCUUU UGCAGUUAGAGAACA	ID. NO. 110
532	UALIAAUGALCUCUUUU QCAGUUAGAGAACAU	ID. NO. 111
538	GAUCUCUUUUGCAGUU AGAGAACAUGGAGAC	ID. NO. 112
539	AUCUCUUUUGCAGUUA GAGAACAUGGAGACU	ID. NO. 113
555	GAGAACAUGGAGACUU UUACCCUUUUGAUGG	ID. NO. 114
556	AGAACAUGGAGACUUU UACOCUUUUGAUGGA	ID. NO. 115
557	GAACAUGGAGACUU ACCCUUUGAUGGAC	ID. NO. 116
558	AACAUGGAGACUUUA COCUUUGAUGGACC	ID. NO. 117
563	GGAGACUUUUACOCUU UUGAUGGACOUGGAA	ID. NO. 118
564	CACACUUUUACOCUUU UGALGGAOCUGGAAA	ID. NO. 119
565	AGACUUUUACCCUUUU GAUGGACCUGGAAAU	ID. NO. 120
583	UGGACCUGGAAAUGUU UUGGCCCAUGCCUAU	ID. NO. 121
584	GCACCUGCAAALGUUU UGGCCCAUGCCUALG	ID. NO. 122
585	CACCUGGAAAUGUUU GGCCCAUGCCUAUGC	ID. NO. 123
597	UUUUGGOOCALGOOLA UGOOOCUGGOOCAGG	ID. NO. 124
616	CCCUGGGCCAGGGAUU AAUGGAGAUGCCCAC	ID. NO. 125
617	CCUGGGCAGGGAUUA AUGGAGAUGCCCACU	ID. NO. 126
<u>හ</u>	AUGGAGAUGOCCACUU UGAUGAUGAUGAACA	ID. NO. 127
634	UCCACALICOCCACUUU CALICALICALICAACAA	ID. NO. 128
662	CAAUGGACAAAGGAUA CAACAGGGACCAAUU	ID. NO. 129
<i>ണ</i>	ACAACAGGGACCAALIU UAUUUCUGGUGGUG	ID. NO. 130
678 678	CAACAGGGACCAAUUU AUUUCUGGUUGCUGC	ID. NO. 131
679	AACAGGGACCAALIULA UUUCUCGUUGCUCCU	ID. NO. 132

681	CAGGGACCAAUUUAUU UCUOGUUGCUGCUCA	ID. NO. 133
682	AGGGACCAAUUUAUUU CUCGUUGCUCAU	ID. NO. 134
683	GOGACCAAUUUAUUC UCGUUGCUGCUCAUG	ID. NO. 135
685	GACCAAUUIAUUUCUC GUUGCUGCUCAUGAA	ID. NO. 136
688	CAAUUUAUUUCUCGUU GCUGCUCAUGAAAUU	ID. NO. 137
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723	ACUCCCUGGGUCUCUU UCACUCAGCCAACAC	ID. NO. 143
724	CICCOIGGGICICIUI CACICAGCAACACI	ID. NO. 144
725	UCCCUGGGUCUCUUC ACUCAGCCAACACUG	ID. NO. 145
729	UGGGUCUCUUCACUC AGCCAACACUGAAGC	ID. NO. 146
746	GCCAACACUGAAGCUU UGAUGUACCCACUCU	ID. NO. 147
7 47	CCAACACUGAAGCUUU GAUGUACCCACUCUA	ID. NO. 148
753	CUGAAGCUUGAUGIA COCACUCIIAUCACUC	ID. NO. 149
760	UUUGAUGUACUCACUC IIAUCACUCACUCACA	ID. NO. 150
762	UGAUGUACOCACUCIA UCACUCACUCACAGA	ID. NO. 151
764	AUGUACCCACUCUAUC ACUCACUCACAGACC	ID. NO. 152
768	ACCCACUCUAUCACUC ACUCACAGACCUGAC	ID. NO. 153
772	ACUCUAUCACUCACUC ACAGACCUGACUCGG	ID. NO. 154
785	CACACACACATEVACAC GRANCOSCOTANCATA	ID. NO. 155
789	CAGACCUGACUCGGUU CCGCCUGUCUCAAGA	ID. NO. 156
790	AGACCUGACUCIGUUC CGCCUGUCUCAAGAU	ID. NO. 157
798	CUCGGUUCCGCCUGUC UCAAGAUGAUAUAAA	ID. NO. 158
800	OGGUUCOGOCUGUCUC AAGAUGAUAUAAAUG	ID. NO. 159
809	CUGUCUCAAGAUGAUA UAAAUGGCALUCAGU	ID. NO. 160
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820	UGALIALIAAAUGGCALU CAGUCCCUCUALIGGA	ID. NO. 162
821	GALIALIAAAUGGCAUUC AGUCCCUCLIALIGGAC	ID. NO. 163
825	UAAAUGGCAUUCAGUC CCUCUAUGGACCUCC	ID. NO. 164
829	DESCRIPCIAGUECCOC FINITESPACES DE COCCESA	ID. NO. 165
831	GCALUCAGUCCUCUTA UGGACCUCCCCUGA	ID. NO. 166
839	COCCUCACION COCCUCACIONOCUS	ID. NO. 167
849	GACCUCCOCUGACUC CCCUGAGACCCCCU	ID. NO. 168

947 GAUGCUGUCAGCACUC UGAGGGGAGAAAUCC 961 UCUGAGGGAGAAAUC CUGAUCUUTAAAGAC 967 GGGAGAAAUCCUGAUC UUUAAAGACAGGCAC 969 GAGAAAUCCUGAUCUU UAAAGACAGGCACUU 970 AGAAAUCCUGAUCUUU AAAGACAGGCACUUU 971 GAAAUCCUGAUCUUUA AAGACAGGCACUUUU UUAAAGACAGGCACUU UUGGCGCAAAUCCCU

GUCCUUUGAUGCUGUC AGCACUCUGAGGGGA

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1025

984 985 UAAAGACAGGCACUUU UGGCGCAAAUCCCUC 986 AAAGACAGGCACUUUU GGGGCAAAUCCCUCA 996 ACUUUUGGGGCAAAUC CCUCAGFAAGCUUGA 1000 UUGGCGCAAAUCCCUC AGGAAG JUGAACCU 1009 AUCCCUCAGGAAGCUU GAACCUGAALUGCAU 1020 AGCUUGAACCUGAAUU GCALUUGALCUCUUC

GAACCUGAALUGCALU UGALCUCUUCALUUU 1026 AACCUGAAUUGCAUUU GAUCUCUUCAUUUUG 1030 UGAAUUGCAUUUGAUC UCUUCAUUUUGGCCA 1032 AALIUGCALUUGAUCUC UUCAUUUUGGCCALC 1034 UUGCALUUGALCUCUU CALUUUGGCCALCUC 1035 UGCAUUGAUCUCUC AUUUUGGCCAUCUCU

1038 AUUUGAUCUCUUCAUU UUGGOCAUCUCUUCC 1039 UUUGALCUCUCALUU UGGCCAUCUCUCCU 1040 UUGALICUCALIUU GGCCALICUCUU 1047 CUUCAUUUUGGCCAUC UCUUCCUCAGGGGU 1049 UCALIUUUGGCCAUCUC UUCCIUCAGGGGUGG

1051 AUUUUGGCCAUCUCUU CCUUCAGGCGUGGAU 1052 UUUUGCCAUCUCUUC CUUCAGGCGUGGALG

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ID. NO. 197 ID. NO. 198

ID. NO. 199 ID. NO. 200

ID. NO. 201 ID. NO. 202 ID. NO. 203

ID. NO. 204

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1055	negocynananacain cyceograegynegae	ID. NO. 205
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1109	CUCGUUUCALUUUUA AAGGAAAUCAALUCU	ID. NO. 220
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1247	AAGAACAAAACAUAUU UCUUUGUAGAGGACA	ID. NO. 239
1248	AGAACAAAACALIALUU CUUUTUAGAGGACAA	ID. NO. 240

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1252	CAAAACAUAUUUCUUU GUAGAGGACAAAUAC	ID. NO. 243
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1293	AUGAGAAGAGAAAUUC CAUGGAGCCAGGCUU	ID. NO. 249
1308	CCAUGGAGCCAGGOUU UCCCAAGCAAALIAGC	ID. NO. 250
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1310	AUGGAGOCAGGCUUC OCAAGCAAAUAGOUG	ID. NO. 252
1321	CUUCCCAAGCAAALIA GCUGAAGACUUUCCA	ID. NO. 253
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1333	AAUAGCUGAAGACUUU CCAGGGAUUGACUCA	ID. NO. 255
1334	AUAGCUGAAGACUUUC CAGGGAUUGACUCAA	ID. NO. 256
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1347	UUCCAGGGAUUGACUC AAAGAUUGAUGCUGU	ID. NO. 258
1354	GAUCCACUCAAAGAUU CAUCCUGUUUUGAA	ID. NO. 259
1363	AAAGALUGAUGCUGUU UUUGAAGAAUUUGG	10. NO. 260
1364	AAGAUUGAUGCUGUUU UUGAAGAAUUUGGGU	ID. NO. 261
1365	AGALUGAUGCUGUUU UGAAGAAUUUGGGUU	ID. NO. 262
1366	GALUGALIGCUGUUUU GAAGAAUUUGGGUUC	ID. NO. 263
1374	CUGUUUUUGAAGAALUU UGGGUUUUUUUUUU	ID. NO. 264
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1381	UGAAGAAUUUGGGUC UUUUAUUUGUUACU	ID. NO. 267
1383	AAGAAUUUGGGUUCUU UUAUUUCUUUACUGG	ID. NO. 268
1384	AGAAUUUGGGUCUUU UAUUUCUUACUGGA	ID. NO. 269
1385	GAALUUGGGUUCUUU ALUUCUUUACUGGAU	ID. NO. 270
1386	AAUUUGGGUUCUUUIA UUUCUUUIACUGGAUC	ID. NO. 271
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1389	UUGGGUCUUUIAUUU CUUUACUGGAUCUUC	ID. NO. 273
1390	UGGGUCUUUIAUUC UUIACUGGALCUCA	ID. NO. 274
1392	GEUCUUUALUUCUU UACUGGALCUCACA	ID. NO. 275
1393	GUUCUUUUAUUUCUUU ACUGGAUCUUCACAG	ID. NO. 276

1394	UUCUUUAUUUCUUUA CUGGAUCUUCACAGU	ID. NO. 277
1401	AUUUCUUUACUGGAUC UUCACAGUUGGAGUU	ID. NO. 278
1403	UUCUUUACUGGAUCUU CACAGUUGGAGUUUG	ID. NO. 279
1404	UCUUUACUGGAUCUUC ACAGUUGGAGUUUGA	ID. NO. 280
1410	CUEGAUCUUCACAGUU GEAGUUUGACOCAAA	ID. NO. 281
1416	CUUCACAGUUGGAGUU UGACOCAAAUGCAAA	ID. NO. 282
1417	UUCACAGUUGGAGUUU GACCCAAAUGCAAAG	ID. NO. 283
1448	AAAGUGACACACUU UGAAGAGUAACAGCU	ID. NO. 284
1449	AAGUGACACACUUU GAAGAGUAACAGCUG	ID. NO. 285
1457	CACACUUUGAAGAGUA ACAGCUGGCUUAAUU	ID. NO. 286
1468	GAGUAACAGCUGGCUU AAUUGUUGAAAGAGA	ID. NO. 287
1469	AGUAACAGCUGGCUUA AUUGUUGAAAGAGAU	ID. NO. 288
1472	AACAGCUGGCUUAAUU GUUGAAAGAGALIAUG	ID. NO. 289
1475	AGCUGGCUUAAUUGUU GAAAGAGAUAUGUAG	ID. NO. 290
1485	AUUSUUGAAAGAGAUA USUAGAAGGCACAAU	ID. NO. 291
1489	UUGAAAGAGALIAUGUA GAAGOCACAALIAUGG	ID. NO. 292
1501	UGUAGAAGOCACAALIA UGGGCACUUUAAAUG	ID. NO. 293
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1511	ACAAUAUGGGCACUUU AAAUGAAGCUAAUAA	ID. NO. 295
1512	CAALIALIGGGCACUULA AALIGAAGCUAALIAALI	ID. NO. 296
1522	ALCOACULUULAALUA ALCOAAGULAAALUUCAC	ID. NO. 297
1525	UEAALCOACUUCULA ALIAALCOAACULAAALU	ID. NO. 298
1528	AAUGAAGCUAAUAAUU CUCACCUAAGUCC	ID. NO. 299
1529	ALIGAAGOUAALIAALUC UUCACOLIAAGUCUCU	ID. NO. 300
1531	GAAGCUAAUAAUUCUU CACCUAAGUCUCUGU	ID. NO. 301
1532	AAGCUAAUAAUUCUUC ACCUAAGUCUCUGUG	ID. NO. 302
1537	AALIAAUUCUUCACCUA AUCOCUUCUGAAUUG	ID. NO. 303
1541	ALTUCULCACCULAAGUC UCUGUGAAAUUGAAAU	ID. NO. 304
1543	UCUCACCUAAGUCUC UGUGAAAUGU	ID. NO. 305
1551	UAAGUCUCUGUGAALU GAAAUGUUCGUUUC	ID. NO. 306
1559	UCCCUCCUUUUUCC UUCUAAAUCUU	ID. NO. 307
1560	GUGAAUUGAAAUGUUC GUUUUCUCCUGCCUG	ID. NO. 308
1563	AAUUGAAAUGUUCGUU UUCUCCUGCCUGUGC	ID. NO. 309
1564	ALIUGAAALGUUGGUUU UCUCCUGCCUGUGU	ID. NO. 310
1565	UUGAAAUGUUGGUUU CUCCUGCUGUGGG	ID. NO. 311
1566	UGAAAUGUUGGUUUC UCCUGCCUGUGCUGU	ID. NO. 312

1568	AANGUUGUUUCUC CUGCUGUGUGAA	ID. NO. 313
1586	GCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ID. NO. 314
1591	UGCUGUGACUCGAGUC ACACUCAAGGGAACU	ID. NO. 315
1597	GACUCGAGUCACACUC AAGGGAACUUGAGCG	ID. NO. 316
1607	ACACUCAAGGGAACUU GAGCGUGAAUCUGUA	ID. NO. 317
1618	AACUUGAGOGUGAAUC UGUAUGUUGCOGGUC	ID. NO. 318
1622	UCAGOGUCAAUCUGUA UCUUGOOGGUCAUUU	ID. NO. 319
1624	AGCGUGAAUCUGUAUC UUGCCGGUCAUUUUU	ID. NO. 320
1626	CECCEVATICACITATICATA GCCCECICATATATATA	ID. NO. 321
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1636	UALICUUGCCGGUCALIU UUUALIGUUALUACAG	ID. NO. 323
1637	AUCUUGOOGGUCALUU UUAUGUUAUUACAGG	ID. NO. 324
1638	UCUUGCOGGUCALUUU UALGULALUACAGGG	ID. NO. 325
1639	CUUGCOGGUCAUUUUU AUGUUAUUACAGGGC	ID. NO. 326
1640	UUGCCGGUCAUUUUA UGUUAUUACAGGCCA	ID. NO. 327
1644	OGGUCALUUULIAUGUU AUUACAGGGCALUCA	ID. NO. 328
1645	GGUCAUUUUIAUGUUA UUACAGGGCAUUCAA	ID. NO. 329
1647	UCAUUUUUAUGUIAUU ACAGGGCAUUCAAAU	ID. NO. 330
1648	CALUUUUALGUUALUA CAGGGCALUCAAALIG	ID. NO. 331
1657	GUUAUUACAGGGCALIU CAAAUGGGGUGCUGC	ID. NO. 3 i2
1658	UUALUACAGGGCALUC AAAUGGGCUGCUGCU	ID. NO. 333
1674	YAYNGGGCTGCTGCTIC YGCTTGCACCTTCCTC	ID. NO. 334
1675	AALOGGCUGCUGCUUA GCUUGCACCUUGUCA	ID. NO. 335
1679	GGCTGCTGCTTAGCTT GCYCCTTCTCACYTY	ID. NO. 336
1686	GCULIAGCUUGCACCUU GUCACALIAGAGUGAU	ID. NO. 337
1689	UAGCUUGCACCUUGUC ACAUAGAGUGAUCUU	ID. NO. 338
1694	UGCACCUUGUCACALIA GAGUGALICUUCCCA	ID. NO. 339
1702	GUCACALIAGAGUGAUC UUUCOCAAGAGAAGG	ID. NO. 340
1704	CACALIAGAGUGALICUU UCCCAAGAGAGGGG	ID. NO. 341
1705	ACALIAGAGUGAUCUUU COCAAGAGAAGGGGA	ID. NO. 342
1706	CALIAGAGUGALUTUUC OCAAGAGAAGGGAA	ID. NO. 343
1727	AGAAGGGGAAGCACUC GUGUGCAACAGACAA	ID. NO. 344
1751	CAGACAAGUGACUGUA UCUGUGUAGACUAUU	ID. NO. 345
1753	GACAAGUGACUGUAUC UGUGUAGACUAUUUG	ID. NO. 346
1759	UCACUGUALUCUGULA GACUALUUGGULIALU	ID. NO. 347
1764	GIALCUGUGUAGACUA UUUGCUUAUUIAALIA	ID. NO. 348

WO 96/18736

Table AIV: Human Stromelysin HP Target Sequence

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Position	Target Sequence Seq.	ID. NO.
66	CANCA CAN CONCACACA	ID. NO. 360
82	UGGCA GUU UGCUCAGCCUIAUCCA	ID. NO. 361
192	AAACA GUU UGUUAGGAGAAAGGA	ID. NO. 362
430	AUGCU GUU GAUUCUGCUGUUGAG	ID. NO. 363
442	CUGCU GUU GAGAAAGCUCUGAAA	ID. NO. 364
775	UCACA GAC CUGACUCGGUUCCCC	ID. NO. 365
1360	AUGCU GUU UUUGAAGAALUUGGG	ID. NO. 366
1407	UCACA GUU GGAGUUUGACCCAAA	ID. NO. 367

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973	AAAGAACA CUGAUGAGGCCGAAAGGCCGAA AAUUUCUC
977	CUUUAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAUU
978	UCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AACAGAAU
980	UGUCUUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAGA
981	CUGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGAACAG
982	CCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AAAGAACA
992	GCCAGAAA, CUGAUGAGGCCGAAAGGCCGAA ACCUGUCU
994	GOGCCAGA CUGAUGAGGCCGAAAGGCCGAA AUACCUGU
995	UGCGCCAG CUGAUGAGGCCGAAAGGCCGAA AAUACCUG
996	UUGCGCCA CUGAUGAGGCCGAAAGGCCGAA AAAUACCU
1007	UCCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGCGC
1011	AGAAUCCU CUGAUGAGGCCGAAAGGCCGAA AGGGACUU
1017	GGUUCGAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGAG
1018	AGGUUCGA CUGAUGAGGCCGAAAGGCCGAA AAUCCUGA
1020	UCAGGUUC CUGAUGAGGCCGAAAGGCCGAA AGAAUCCU
1031	UCAAAUGA CUGAUGAGGCCGAAAGGCCGAA ACUCAGGU
1032	AUCAAAUG CUGAUGAGGCCGAAAGGCCGAA AACUCAGG
1033	GAUCAAAU CUGAUGAGGCCGAAAGGCCGAA AAACUCAG
1036	AGAGAUCA CUGAUGAGGCCGAAAGGCCGAA AUGAAACU
1037	AAGAGAUC CUGAUGAGGCCGAAAGGCCGAA AAUGAAAC
1041	AAUGAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAAAUG
1043	AGAAUGAA CUGAUGAGGCCGAAAGGCCGAA AGAUCAAA
1045	CCAGAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGAUCA
1046	GCCAGAAU CUGAUGAGGCCGAAAGGCCGAA AAGAGAUC
1049	AUGGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGAG
1050	GAUGGCCA CUGAUGAGGCCGAAAGGCCCGAA AAUGAAGA
1058	AAGGAAGA CUGAUGAGGCCGAA AUGGCCAG
1060	UGAAGGAA CUGAUGAGGCCGA: CCCCGAA AGAUGGCC
1062	GCUGAAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAUGG
1063	UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAUG
1066	CACUGCUG CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
1067	CCACUGCU CUGAUGAGGCCGAAAGGCCGAA AAGGAAGA
1085	UAACUUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGCA
1092	CUGCUAAU CUGAUGAGGCCGAAAGGCCGAA ACUUCAUA
1093	CCUGCUAA CUGAUGAGGCCGAAAGGCCGAA AACUUCAU
1095	UCCCUGCU CUGAUGAGGCCGAAAGGCCGAA AUAACUUC
1096	AUCCCUGC CUGAUGAGGCCGAAAGGCCGAA AAUAACUU
1105	GAAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCUGC
1110	AAAAUGAA CUGAUGAGGCCGAAAGGCCGAA ACAGUAUC
1111	AAAAAUGA CUGAUGAGGCCGAAAGGCCGAA AACAGUAU
1112	UAAAAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAGUA
1113	UUAAAAAU CUGAUGAGGCCGAAAGGCCGAA AAAACAGU
1116	CCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AUGAAAAC
1117	UCCUUUAA CUGAUGAGGCCGAAAGGCCGAA AAUGAAAA
1118	UUCCUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUGAAA
1119	GUUCCUUU CUGAUGAGGCCGAAAGGCCGAA AAAAUGAA
1120	AGUUCCUU CUGAUGAGGCCGAAAGGCCGAA AAAAAUGA
1129	CCAGAACU CUGAUGAGGCCGAAAGGCCGAA AGUUCCUU
1133	UGGCCCAG CUGAUGAGGCCGAAAGGCCGAA ACUGAGUU

	•
1134	AUGGCCCA CUGAUGAGGCCGAAAGGCCGAA AACUGAGU
1143	UUUCCUCU CUGAUGAGGCCGAAAGGCCGAA AUGGCCCA
1144	AUUUCCUC CUGAUGAGGCCGAAAGGCCGAA AAUGGCCC
1158	CCAGCUUG CUGAUGAGGCCGAAAGGCCGAA ACCUCAUU
1168	UCUUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGCUU
1169	UUCUUGGG CUGAUGAGGCCGAAAGGCCGAA AACCAGCU
1182	AGGGUGUG CUGAUGAGGCCGAAAGGCCGAA AUGCUUCU
1195	UGAAGGGA CUGAUGAGGCCGAAAGGCCGAA ACCCAGGG
1196	UUGAAGGG CUGAUGAGGCCGAAAGGCCGAA AACCCAGG
1197	GUUGAAGG CUGAUGAGGCCGAAAGGCCGAA AAACCCAG
1201	UAUGGUUG CUGAUGAGGCCGAAAGGCCGAA AGGGAAAC
1202	UUAUGGUU CUGAUGAGGCCGAAAGGCCGAA AAGGGAAA
1209	AUUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUGGUUGA
1218	GCAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUUUUUCU
1230	UUAUCAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCAGC
1231	CUUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUGGCAG
1232	CCUUAUCA CUGAUGAGGCCGAAAGGCCGAA AAAUGGCA
1237	CCUUUCCU CUGAUGAGGCCGAAAGGCCGAA AUCAGAAA
1256	CAAAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGUUUUC
1259	CCACAAAG CUGAUGAGGCCGAAAGGCCGAA AGUAUGUU
1260	UCCACAAA CUGAUGAGGCCGAAAGGCCGAA AAGUAUGU
1262	CUUCCACA CUGAUGAGGCCGAAAGGCCGAA AGAAGUAU
1263	UCUUCCAC CUGAUGAGGCCGAAAGGCCGAA AAGAAGUA
1277	ACCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUCU
1286	UCUCAUCA CUGAUGAGGCCGAAAGGCCGAA ACCUCCAG
1287	UUCUCAUC CUGAUGAGGCCGAAAGGCCGAA AACCUCCA
1304	GCUCCAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUCUC
1319	GUCUGGGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGGC
1320	UGUCUGGG CUGAUGAGGCCGAAAGGCCCGAA AAGCCUGG
1321	AUGUCUGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCUG
1330	UUCUGCUA CUGAUGAGGCCGAAAGGCCGAA AUGUCUGG
1332	UCUUCUGC CUGAUGAGGCCGAAAGGCCGAA AUAUGUCU
1343	UUCCUGGA CUGAUGAGGCCGAAAGGCCGAA AGUCUUCU
1344	AUUCCUGG CUGAUGAGGCCGAAAGGCCGAA AAGUCUUC
1345	AAUUCCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCUU
1353	UUUGGAUU CUGAUGAGGCCGAAAGGCCGAA AUUCCUGG
1354	CUUUGGAU CUGAUGAGGCCGAAAGGCCGAA AAUUCCUG
1357	GAUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUAAUUC
1365	ACAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUGG
1374 1375	GCUUCAAA CUGAUGAGGCCGAAAGGCCGAA ACAGCAUC
1376	UGCUUCAA CUGAUGAGGCCGAAAGGCCGAA AACAGCAU
1377	AUGCUUCA CUGAUGAGGCCGAAAGGCCGAA AAACAGCA
1385	AAUGCUUC CUGAUGAGGCCGAAAGGCCGAA AAAACAGC
1386	AAAACCCA CUGAUGAGGCCGAAAGGCCGAA AUGCUUCA
1391	AAAAACCC CUGAUGAGGCCGAAAGGCCGAA AAUGCUUC
1391	AAUAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCCAAAU
1392	AAAUAGAA CUGAUGAGGCCGAAAGGCCGAA AACCCAAA
1393	GAAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCAA
4J 7 4	AGAAAUAG CUGAUGAGGCCGAAAGGCCGAA AAAACCCA

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1395		CUGAUGAGGCCGAAAGGCCGAA	
1397	UGAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAAC
1399	ACUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAAAA
1400	CACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAAA
1401	CCACUGAA	CUGAUGAGGCCGAAAGGCCCGAA	AAAUAGAA
1403	AUCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUAG
1404	GAUCCACU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAUA
1412	ACUGUGAA	CUGAUGAGGCCGAAAGGCCCGAA	AUCCACUG
1414	CGACUGUG	CUGAUGAGGCCGAAAGGCCCGAA	AGAUCCAC
1415	CCGACUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCCA
1421	CAAACUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGAA
1427	UUGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGAC
1428	UUUGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AACUCCGA
1458	CUCUUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUGU
1459	GCUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGUG
1460	UGCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUGU
1478	AACACUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUG
1479	UAACACUG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1480	CUAACACU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAGC
1486	ccccccca	CUGAUGAGGCCGAAAGGCCGAA	ACACUGAA
1487	ACCCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AACACUGA
1498	GCCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	ACACCCCU
1500	GUGCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUACACCC
1519	UCAUUUAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCAU
1520	UUCAUUUA	CUGAUGAGGCCGAAAGGCCCGAA	AACAUUCA
1521	GUUCAUUU	CUGAUGAGGCCGAAAGGCCCGAA	AAACAUUC
1522	GGUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AA? "CAUU
1532	UGAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AG. OCAU
1535	UGUUGAAC	CUGAUGAGGCCGAAAGGCCCGAA	AUUAGGUU
1538		CUGAUGAGGCCGAAAGGCCGAA	
1539	UAAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AACAAUUA
1546	AAAGUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUGA
1547	CAAAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUUG
1553	AACUCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUAA
1554	CAACUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCUA
1561	GCCACUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCACAA
1571	GAGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACUU
1574	CAGGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCCA
1575	GCAGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGCC
1576	UGCAGGAG	CUGAUGAGGCCGAAAGGCCCGAA	AAAUGAGC
157 7		CUGAUGAGGCCGAAAGGCCCGAA	
1579	AUAUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAUG
1586		CUGAUGAGGCCGAAAGGCCCGAA	
1602		CUGAUGAGGCCGAAAGGCCGAA	
1604		CUGAUGAGGCCGAAAGGCCGAA	
1620		CUGAUGAGGCCGAAAGGCCGAA	
1622		CUGAUGAGGCCGAAAGGCCGAA	
1624		CUGAUGAGGCCGAAAGGCCGAA	
1633	GAUGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAGU

1634	CGAUGUCC CUGAUGAGGCCGAAAAGGCCGAA AAGUCCAG
1641	CCCGUAAC CUGAUGAGGCCGAAAGGCCGAA AUGUGCAA
1644	ACACCCGU CUGAUGAGGCCGAAAGGCCGAA ACGAUGUG
1645	AACACCCG CUGAUGAGGCCGAAAGGCCGAA AACGAUGU
1653	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA ACACCCGU
1654	GCCUGUUU CUGAUGAGGCCGAAAAGGCCGAA AACACCCG
1670	UGCAAGCU CUGAUGAGGCCGAAAGGCCGAA AGCAGCAG
1671	GUGCAAGC CUGAUGAGGCCGAAAAGGCCGAA AAGCAGCA
1675	UCAAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCUAAGC
1681	AUGUGAUC CUGAUGAGGCCGAAAGGCCGAA AGUGCAAG
1685	UUCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUCAAGUG
1701	UCUCGUGG CUGAUGAGGCCGAAAAGGCCGAA AGCUCCCU
1702	GUCUCGUG CUGAUGAGGCCGAAAAGCCCGAA AAGCUCCC
1720	CACAUGAG CUGAUGAGGCCGAAAAGGCCGAA ACUUCCCC
1723	UCACACAU CUGAUGAGGCCGAAAGGCCGAA AGUACUUC
1744	AUAGACAC CUGAUGAGGCCGAAAAGGCCGAA AUCACUCG
1749	UCCACAUA CUGAUGAGGCCGAAAGGCCGAA ACACAAUC
1751	AAUCCACA CUGAUGAGGCCGAAAGGCCGAA AGACACAA
1759	GGGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUCCACAU
1760	UGGGCAAA CUGAUGAGGCCGAAAAGGCCGAA AAUCCACA
1762	AAUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUAAUCCA
1763	UAAUGGGC CUGAUGAGGCCGAAAGGCCGAA AAUAAUCCA
1770	UAUUAAAU CUGAUGAGGCCGAAAGGCCGAA AUGAGCAA
1771	UUAUUAAA CUGAUGAGGCCGAAAGGCCGAA AAUGGGCA
1773	CUITIAITIA CIGAINTACCOCCA ACCOCCA AAUGGGCA
1774	CUUUAUUA CUGAUGAGGCCGAAAGGCCGAA AUAAUGGG
1775	UCUUUAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAUGG
· 72	CUCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAUG
1787	AUCCUCUU CUGAUGAGGCCGAAAGGCCGAA AUUAAAUA
	AAUUGACA CUGAUGAGGCCGAAAGGCCGAA AUCCUCUU

Table AVIII: Human Stromelysin Hairpin Ribozyme and Target Sequences

nt.	RZ	Substrate
Position		
99	CGCACAGC AGAA GUAGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	uccuacu guu ocuguece
82	GUGAGCA AGAA OCCACG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	
91	ANUGGAUA AGAA GACCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCUCA GCC UAUCCAUU
192	UCCURACA AGRA GUIUCA ACCAGAGAAACACACGUUGUGGGAACAUUACCUGGUA	3
220	UUUUVAAC AGAA GGACCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGUCCU GUU GUUAAAAA
328	VGACCAAC AGAA GGAACU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGUUCCU GAU GUUGGUCA
412	UUUGGCAA AGAA GGUGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UACACCA GAU UUGCCAAA
430	GCAGAAUC AGAA GCAUCU ACCAGAGAAACACACGUUGUOGUACAUUACCUGGUA	AGAUGCU GUU GAUUCUGC
439	UUCUCAAC AGAA GAAUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGAUUCU GCU GUUGAGAA
442	GCUUNCUC AGAA GCAGAA ACCAGAGAAACACACGUUCUGGUACAUUACCUGGUA	3
691	AUTUCAUG AGAA GCAACG ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA	g
775	CGAGUCAG AGAA GUGAGU ACCAGAGAAACACACGUUGUGGUGGUACAUUACCUGGUA	S
780	GGAACCGA AGAA GGUCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	
786	ACAGGGG AGAA GAGUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	35
791	UVGAGACA AGAA GAACCG ACCAGAGAAACACACGUUGUGGUAGAUUACCUGGUA	COGULCE GCC LIGUICAA
795	CAUCTUGA AGAA GGCGGA ACCAGAGAAACACACGTUGTGGTJACAUTJACCTGGTJA	ucceccu auc ucaagaug
822	CAUAGAGG AGAA GAAUGC ACCAGAGAAACACACGUUGUGUGUAGUACAUUACCUGGUA	GCAUDCA GUC CCUCUAUG
844	UCAGGGGA AGAA GGGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCCCCU GAC UCCCCUGA
880	UCUGGAGG AGAA GGUUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAACCU GUC CCUCCAGA
919	AGA	UGAUCCU GCU UUGUCCUU
963	CIUDAAAG AGAA GGAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAUCCU GAU CUUUAAAG
1360	UCTUCAAA AGAA GCAUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGAUGCU GUU UUUGAAGA
1407	ACTA	CUDCACA GUU GCAGUUUG
1460	ACA	AGUAACA GCU GGCUUAAU
1570	ACAGCACA AGAA GGAGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	התכתכנת פכב תפתפכתפת
1667	AGCUAAGC AGAA GCCCAU ACCAGAGAAACACACGUGGUGGUACAUUACCUGGUA	AUGGGCU GCU GCUUAGCU
1670	GCAAGCUA AGAA GCAGCC ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GOCUGCU GCU UAGCUUGC

Substrate

Table AIX: Rabbit Hairpin Ribozyme and Target Sequences

Ribozyme Sequence

nt. Position

UDATACO OCU GUBCEUCE	UDGUCA OCC UMUCCACU	USARACA GUU UGUDARAR	UPGUCCU GUU GUURARAA	COUNCY CAU CUCCUCA	CACACOG GAU CUCCCAAG	AGAUDECU GUU GAUDECUDE	8	uscatcu cau ucacucas	AMBOOGOU CAU CURCOCCMG	GIRCOCH GUC URCARGOC	CUCACA GAC CUCACCCC	usacces and coaccium	8	8	GEOCOCU GCC UCUCCUEA	g	3		AAAUUCU GUU CUUUNAAG		GANCIER OU CLOSSECA			CUCACA CUC COACUUC	ACCAACA CCU CCUUCAG	ACAGGCU GCU GCUUAGCU	accused acu uraccuse	USICACA CAC CACICAUU
		E.A.				AG.	G	¥ Q	AGA A	A CA	¥ Q	COS AGNA GOSCCA ACCACACACACACACACOSTOCOSTACAUTACCUCICIA	AC SA		AGNA GOGGCC	K.	AGNA GGAUCA	AGNA GALICUG	ACAA CAAUUU	AGNA GUALUCC	AGA AGA	AGNA GUCUCU	AGNA GCAUCG	AGNA GUGNAG	Q.			IC AGNA GICACA ACCAGAGAAACACGICGICGIGGIACAUUACCICGIA
	~	203 UUUUPACA	_	339 CARCONAC				•	758 CUGGGUAC	•	786 (33302)6			•	_	•	930 AAGGACAG		_	~		1301 OCUCCAGO			•			1733 AAUCACUC

Table BII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AAACCCU C UGUAAAG	236	UGUGUGU U UUGUAAA
12	CCUCUGU A AAGUAAC	237	GUGUGUU U UGUAAAC
17	GUAAAGU A ACAGAAG	238	UGUGUUU U GUAAACA
26	CAGAAGU U AGAAGGG	241	GUUUUGU A AACAUCA
27	AGAAGUU A GAAGGGG	247	UAAACAU C ACUGGAG
41	GAAAUGU C GCCUCUC	258	GGAGGGU C UUCUACG
46	GUCGCCU C UCUGAAG	260	AGGGUCU U CUACGUG
48	CGCCUCU C UGAAGAU	261	GGGUCUU C UACGUGA
56	UGAAGAU U ACCCAAA	263	GUCUUCU A CGUGAGC
57	GAAGAUU A CCCAAAG	274	GAGCAAU U GGAUUGU
75	AAGUGAU U UGUCAUU	279	AUUGGAU U GUCAUCA
76	AGUGAUU U GUCAUUG	. 282	GGAUUGU C AUCAGCC
7 9	GAUUUGU C AUUGCUU	285	UUGUCAU C AGCCCUG
82	UUGUCAU U GCUUUAU	298	UGCCUGU U UUGCACC
86	CAUUGCU U UAUAGAC	299	GCCUGUU U UGCACCU
87	AUUGCUU U AUAGACU	300	CCUGUUU U GCACCUG
88	UUGCUUU A UAGACUG	322 -	CCCUGGU C UUACUUG
90	GCUUUAU A GACUGUA	. 324	CUGGUÇU U ACUUGGG
97	AGACUGU A AGAAGAG	325	UGGUCUU A CUUGGGU
110	AGAACAU C UCAGAAG	328	UCUUACU U GGGUCCA
112	AACAUCU C AGAAGUG	333	CUUGGGU C CAAAUUG
124	GUGGAGU C UUACCCU	339	UCCAAAU U GUUGGCU
126	GGAGUCU U ACCCUGA	342	AAAUUGU U GGCUUUC
127	GAGUCUU A CCCUGAA	347	GUUGGCU U UCACUUU
137	CUGAAAU C AAAGGAU	348	UUGGCUU U CACUUUU
145	AAAGGAU U UAAAGAA	349	UGGCUUU C ACUUUUG
146	AAGGAUU U AAAGAAA	353	UUUCACU U UUGACCC
147	AGGAUUU A AAGAAAA	354	UUCACUU U UGACCCU
163	GUGGAAU U UUUCUUC	355	UCACUUU U GACCCUA
164	UGGAAUU U UUCUUCA	362	UGACCCU A AGCAUCU
165	GGAAUUU U UCUUCAG	368	UAAGCAU C UGAAGCC
166	GAAUUUU U CUUCAGC	404	GGAACAU C ACCAUCC
167	AAUUUUU C UUCAGCA	410	UCACCAU C CAAGUGU
169	UUUUUCU U CAGCAAG	418	CAAGUGU C CAUACCU
170	UUUUCUU C AGCAAGC	422	UGUCCAU A CCUCAAU
187 191	UGAAACU A AAUCCAC	426	CAUACCU C AAUUUCU
200	ACUAAAU C CACAACC	430	CCUCAAU U UCUUUCA
	ACAACCU U UGGAGAC	431	CUCAAUU U CUUUCAG
201	CAACCUU U GGAGACC	432	UCAADUU C UUUCAGC
221 226	ACACCCU C CAAUCUC	434	AAUUUCU U UCAGCUC
	CUCCAAU C UCUGUGU	435	AUUUCUU U CAGCUCU
228	CCAAUCU C UGUGUGU	436	UUUCUUU C AGCUCUU

441	UUCAGCU C UUGGUGC	782	GENORI II MANAGA
443	CAGCUCU U GGUGCUG	783	GUGACGU U AUCAGUC
457	GGCUGGU C UUUCUCA	785	UGACGUU A UCAGUCA ACGUUAU C AGUCAAA
459	CUGGUCU U UCUCACU	789	UAUCAGU C AAAGCUG
460	UGGUCUU U CUCACUU	800	
461	GGUCUUU C UCACUUC	801	GCUGACU U CCCUACA
463	UCUUUCU C ACUUCUG	805	CUGACUU C CCUACAC
467	UCUCACU U CUGUUCA	811	CUUCCCU A CACCUAG
468	CUCACUU C UGUUCAG	814	UACACCU A GUAUAUC
472	CUUCUGU U CAGGUGU	816	ACCUAGU A UAUCUGA
473	UUCUGUU C AGGUGUU	818	CUAGUAU A UCUGACU AGUAUAU C UGACUUU
480	CAGGUGU U AUCCACG	824	
481	AGGUGUU A UCCACGU	825	UCUGACU U UGAAAUU
483	GUGUUAU C CACGUGA	831	CUGACUU U GAAAUUC
521	. ACGCUGU C CUGUGGU	832	UUGAAAU U CCAACUU
529	CUGUGGU C ACAAUGU	838	UGAAAUU C CAACUUC
537	ACAAUGU U UCUGUUG	839	UCCAACU U CUAAUAU
538	CAAUGUU U CUGUUGA	841	CCAACUU C UAAUAUU AACUUCU A AUAUUAG
539	AAUGUUU C UGUUGAA	844	UUCUAAU A UUAGAAG
543	UUUCUGU U GAAGAGC	846	CUAAUAU U AGAAGGA
562	ACAAACU C GCAUCUA	847	UAAUAUU A GAAGGAU
567	CUCGCAU C UACUGGC	855	GAAGGAU A AUUUGCU
569	CGCAUCU A CUGGCAA	858	GGAUAAU U UGCUCAA
601	GCUGACU A UGAUGUC	859	GAUAAUU U GCUCAAC
608	AUGAUGU C UGGGGAC	863	
622	CAUGAAU A UAUGGCC	869	AUUUGCU C AACCUCU UCAACCU C UGGAGGU
624	UGAAUAU A UCGCCCG	877	UGGAGGU U UUCCAGA
635	CCCGAGU A C WAAAC	878	GGAGGUU U UCCAGAG
651	GGACCAU C UUUGAUA	879	GAGGUUU U CCAGAGC
653	ACCAUCU U UGAUAUC	880	AGGUUUU C CAGAGCC
654	CCAUCUU U GAUAUCA	889	AGAGCCU C ACCUCUC
658	CUUUGAU A UCACUAA	894	CUCACCU C UCCUGGU
660	UUGAUAU C ACUAAUA	896	CACCUCU C CUGGUUG
664	UAUCACU A AUAACCU	902	UCCUGGU U GGAAAAU
667	CACUAAU A ACCUCUC	920	GAAGAAU U AAAUGCC
672	AUAACCU C UCCAUUG	921	AAGAAUU A AAUGCCA
674	AACCUCU C CAUUGUG	930	AUGCCAU C AACACAA
678	UCUCCAU U GUGAUCC	942	CAACAGU U UCCCAAG
684	UUGUGAU C CUGGCUC	943	AACAGUU U CCCAAGA
691	CCACCCA C ACCCCCC	944	ACAGUUU C CCAAGAU
701	CGCCCAU C UGACGAG	952	CCAAGAU C CUGAAAC
716	GGCACAU A CGAGUGU	966	CUGAGCU C UAUGCUG
726	AGUGUGU U GUUCUGA	968	GAGCUCU A UGCUGUU
729	GUGUUGU U CUGAAGU	975	AUGCUGU U AGCAGCA
730	UGUUGUU C UGAAGUA	976	UGCUGUU A GCAGCAA
737	CUGAAGU A UGAAAAA	991	ACUGGAU U UCAAUAU
751	AGACGCU U UCAAGCG	992	CUGGAUU U CAAUAUG
752	GACGCUU U CAAGCGG	993	UGGAUTU C AAUAUGA
753	ACGCUUU C AAGCGGG	997	UUUCAAU A UGACAAC
			SOUTH A WALARC

1016	CACAGCU U CAUGUGU	1315	CAUGGAU C GUGGGGA
1017	ACAGCUU C AUGUGUC	1324	UGGGGAU C AUGAGGC
1024	CAUGUGU C UCAUCAA	1334	GAGGCAU U CUUCCCU
1026	UGUGUCU C AUCAAGU	1335	AGGCAUU C UUCCCUU
1029	GUCUCAU C AAGUAUG	1337	GCAUUCU U CCCUUAA
1034	AUCAAGU A UGGACAU	1338	CAUUCUU C CCUUAAC
1042	UGGACAU U UAAGAGU	1342	CUUCCCU U AACAAAU
1043	GGACAUU U AAGAGUG	1343	UUCCCUU A ACAAAUU
1044	GACAUUU A AGAGUGA	1350	AACAAAU U UAAGCUG
1054	AGUGAAU C AGACCUU	1351	ACAAAUU U AAGCUGU
1061	CAGACCU U CAACUGG	1352	CAAAUUU A AGCUGUU
1062	AGACCUU C AACUGGA	1359	AAGCUGU U UUACCCA
1072	CUGGAAU A CAACCAA	1360	AGCUGUU U UACCCAC
1090	AGAGCAU U UUCCUGA	1361	GCUGUUU U ACCCACU
1091	GAGCAUU U UCCUGAU	1362	CUGUUUU A CCCACUA
1092	AGCAUUU U CCUGAUA	1369	ACCCACU A CCUCACC
1093	GCAUUUU C CUGAUAA	1373	ACUACCU C ACCUUCU
1099	UCCUGAU A ACCUGCU	1378	CUCACCU U CUUAAAA
1107	ACCUGCU C CCAUCCU	1379	UCACCUU C UUAAAAA
1112	CUCCCAU C CUGGGCC	1381	ACCUUCU U AAAAACC
1122	GGGCCAU U ACCUUAA	1382	CCUUCUU A AAAACCU
1123	GGCCAUU A CCUUAAU	1390	AAAACCU C UUUCAGA
1127	AUUACCU U AAUCUCA	1392	AACCUCU U UCAGAUU
1128	UUACCUU A AUCUCAG	1393	ACCUCUU U CAGAUUA
1131	CCUUAAU C UCAGUAA	1394	CCUCUUU C AGAUUAA
1133	UUAAUCU C AGUAAAU	1399	UUCAGAU U AAGCUGA
1137	UCUCAGU A AAUGGAA	1400	UCAGAUU A AGCUGAA
1146	AUGGAAU U UUUGUGA	1412	GAACAGU U ACAAGAU
1147	UGGAAUU U UUGUGAU	1413	AACAGUU A CAAGAUG
1148	GGAAUUU U UGUGAUA	1429	CUGGCAU C CCUCUCC
1149	GAAUUUU U GUGAUAU	1433	CAUCCCU C UCCUTUC
1155	UUGUGAU A UGCUGCC	1435	nccenen e cannane
1169	CUGACCU A CUGCUUU	1438	בתבתבבת ה הבתבכבב
1175	UACUGCU U UGCCCCA	1439	UCUCCUU U CUCCCCA
1176	ACUGCUU U GCCCCAA	1440	CUCCUUU C UCCCCAU
1214	GAGAGAU U GAGAAGG	1442	CCUUUCU C CCCAUAU
1230	AAAGUGU A CGCCCUG	1448	UCCCCAU A UGCAAUU
1239	GCCCUGU A UAACAGU	1455	AUGCAAU U UGCUUAA
1241	CCUGUAU A ACAGUGU	1456	UGCAAUU U GCUUAAU
1249	ACAGUGU C CGCAGAA	1460	AUUUGCU U AAUGUAA
1275	AAAAGAU C UGAAGGU	1461	UUUGCUU A AUGUAAC
1283	UGAAGGU A GCCUCCG	1466	UUAAUGU A ACCUCUU
1288	GUAGCCU C CGUCAUC	1471	GUAACCU C UUCUUUU
1292	CCUCCGU C AUCUCUU	1473	AACCUCU U CUUUUGC
1295	CCGUCAU C UCUUCUG	1474	ACCUCUU C UUUUGCC
1297	GUCAUCU C UUCUGGG	1476	CUCUUCU U UUGCCAU
1299	CAUCUCU U CUGGGAU	1477	UCUUCUU U UGCCAUG
1300	AUCUCUU C UGGGAUA	1478	CUUCUUJ U GCCAUGU
1307	CUGGGAU A CAUGGAU	1486	GCCAUGU U CCCAUUC
			THE PROPERTY OF THE PROPERTY O

1487	CCAUGUU U CCAUUCU
1488	CAUGUUU C CAUUCUG
1492	UUUCCAU U CUGCCAU
1493	UUCCAUU C UGCCAUC
1500	CUGCCAU C UUGAALIU
1502	GCCAUCU U GAAUUGU
1507	CUUGAAU U GUCUUGU
1510	GAAUUGU C UUGUCAG
1512	AUUGUCU U GUCAGCC
1515	GUCUUGU C AGCCAAU
1523	AGCCAAU U CAUUAUC
1524	GCCAAUU C AUUAUCU
1527	AAUUCAU U AUCUAUU
1528	AUUCAUU A UCUAUUA
1530	UCAUUAU C UAUUAAA
1532	AUUAUCU A UUAAACA
1534	UAUCUAU U AAACACU
1535	AUCUAUU A AACACUA
1542	AAACACU A AIRINGAG

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Table BIII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	CUUUACA CUGAUGAGGCCGAAAGGCCGAA AGGGUUU
12	GUUACUU CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
17	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACUUUAC
26	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
27	CCCCUUC CUGAUGAGGCCGAAAGGCCGAA AACUUCU
41	GAGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAUUUC
46	CUUCAGA CUGAUGAGGCCGAAAGGCCGAA AGGCGAC
48	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGAGGCG
56	UUUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
57	CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
75	AAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCACUU
76	CAAUGAC CUGAUGAGGCCGAAAGGCCGAA AAUCACU
79	AAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAAAUC
82	AUAAAGC CUGAUGAGGCCGAAAGGCCGAA AUGACAA
86	GUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGCAAUG
87	AGUCUAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAU
88	CAGUCUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
90	UACAGUC CUGAUGAGGCCGAAAGGCCGAA AUAAACC
97	CUCUUCU CUGAUGAGGCCGAAAGGCCGAA ACAGUCU
110	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUGUÜCU
112	CACUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGUU
124	AGGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCCAC
126	UCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGACUCC
127	UUCAGGG CUGAUGAGGCCGAAAGGCCGAA AAGACUC
137 145	AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
145	UUCUUUA CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
147	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA AAUCCUU
163	UUUUCUU CUGAUGAGGCCGAAAGGCCGAA AAAUCCU
164	GAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUCCAC
165	UGAAGAA CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
166	CUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAUUCC
167	GCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAAAUUC
169	UGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAUU
170	CUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAAA GCUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAAA
187	GUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
191	GGUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
200	GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGU
201	GGUCUCC CUGAUGAGGCCGAAAAGGCCGAA AAGGUUG
221	GAGAUUG CUGAUGAGGCCGAAAAGGCCGAA AAGGUUG
226	ACACAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGAG

228	ACACACA CUGAUGAGGCCGAAAGGCCGAA	AGAUUGG
236	UUUACAA CUGAUGAGGCCGAAAGGCCGAA	ACACACA
237	GUUUACA CUGAUGAGGCCGAAAGGCCGAA	AACACAC
238	UGUUUAC CUGAUGAGGCCGAAAGGCCGAA	AAACACA
241	UGAUGUU CUGAUGAGGCCGAAAGGCCGAA	ACAAAAC
247	CUCCAGU CUGAUGAGGCCGAAAGGCCGAA	AUGUUUA
258	CGUAGAA CUGAUGAGGCCGAAAGGCCGAA	ACCCUCC
260	CACGUAG CUGAUGAGGCCGAAAGGCCGAA	AGACCCU
261	UCACGUA CUGAUGAGGCCGAAAGGCCGAA	AAGACCC
263	GCUCACG CUGAUGAGGCCGAAAGGCCGAA	AGAAGAC
274	ACAAUCC CUGAUGAGGCCGAAAGGCCGAA	AUUGCUC
279	UGAUGAC CUGAUGAGGCCGAAAGGCCGAA	AUCCAAU
282	GGCUGAU CUGAUGAGGCCGAAAGGCCGAA	ACAAUCC
285	CAGGGCU CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
298	GGUGCAA CUGAUGAGGCCGAAAGGCCGAA	ACAGGCA
299	AGGUGCA CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
300	CAGGUGC CUGAUGAGGCCGAAAGGCCCGAA	AAACAGG
322	CAAGUAA CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
324	CCCAAGU CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
325	ACCCAAG CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
328	UGGACCC CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
333	CAAUUUG CUGAUGAGGCCGAAAGGCCGAA	ACCCAAG
339	AGCCAAC CUGAUGAGGCCGAAAGGCCGAA	AUUUGGA
342	GAAAGCC CUGAUGAGGCCGAAAGGCCGAA	ACAAUUU
347	AAAGUGA CUGAUGAGGCCGAAAGGCCGAA	AGCCAAC
348	AAAAGUG CUGAUGAGGCCGAAAGGCCGAA	AAGCCAA
349	CAAAAGU CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
353		AGUGAAA
354	AGGGUCA CUGAUGAGGCCGAAAGGCCGAA	AAGUGAA
355	UAGGGUC CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
362	AGAUGCU CUGAUGAGGCCGAAAGGCCGAA	AGGGUCA
368	GGCTUCA CUGAUGAGGCCGAAAGGCCGAA	AUGCUUA
404	GGAUGGU CUGAUGAGGCCGAAAGGCCGAA	AUGUUCC
410	ACACUUG CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
418	AGGUAUG CUGAUGAGGCCGAAAGGCCGAA	ACACUUG
422	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA	AUGGACA
426	AGAAAUU CUGAUGAGGCCGAAAGGCCGAA	AGGUAUG
430	UGAAAGA CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
431	CUGAAAG CUGAUGAGGCCGAAAGGCCGAA	AAUUGAG
432	GCUGAAA CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
434	GAGCUGA CUGAUGAGGCCGAAAGGCCGAA A	AGAAAUU
435	AGAGCUG CUGAUGAGGCCGAAAGGCCGAA	LAGAADA
436	. AAGAGCU CUGAUGAGGCCGAA A	AAAGAAA
441 443	GCACCAA CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
-	CAGCACC CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
457	UGAGAAA CUGAUGAGGCCGAAAGGCCGAA A	ACCAGCC
459	AGUGAGA CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
460	AAGUGAG CUGAUGAGGCCGAAAGGCCGAA A	NAGACC A
461	GAAGUGA CUGAUGAGGCCGAAAGGCCGAA A	AAGACC

463	CAGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGA
467	UGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AGUGAGA
468	CUGAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAG
472	ACACCUG	CUGAUGAGGCCGAAAGGCCCGAA	ACAGAAG
473	AACACCU	CUGAUGAGGCCGAAAGGCCGAA	AACAGAA
480	CGUGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACACCUG
481	ACGUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACACCU
483	UCACGUG	CUGAUGAGGCCGAAAGGCCGAA	AUAACAC
521	ACCACAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCGU
529	ACAUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACCACAG
537	CAACAGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
538	UCAACAG	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
539	UUCAACA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUU
543	GCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAA
562	UAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUGU
567	GCCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAG
569	UUGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
601	GACAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGO
608	GUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAU
622	GGCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAUG
624	CGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUCA
635	GUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGG
651	UAUCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUCC
653	GAUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGU
654	UGAUAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGG
658 ·	UUAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAG
660	UAUUAGU	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAA
664	AGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGAUA
667	GAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGUG
672		CUGAUGAGGCCGAAAGGCCGAA	
674	CACAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
678	G VAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGA
684	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
691	GGGCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
701		CUGAUGAGGCCGAAAGGCCGAA	
716	ACACUCG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGCC
726	UCAGAAC	CUGAUGAGGCCGAAAGGCCGAA	ACACACU
729	ACCUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACAACAC
730		CUGAUGAGGCCGAAAGGCCGAA	
737		CUGAUGAGGCCGAAAGGCCGAA	
751		CUGAUGAGGCCGAAAGGCCGAA	
752		CUGAUGAGGCCGAAAGGCCCGAA	
753		CUGAUGAGGCCGAAAGGCCGAA	
782		CUGAUGAGGCCGAAAGGCCCGAA	
783		CUGAUGAGGCCGAA	
785		CUGAUGAGGCCGAAAGGCCGAA	
789		CUGAUGAGGCCGAAAGGCCCGAA	
800		CUGAUGAGGCCGAAAGGCCGAA	
801	GUGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG

805	CUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAG
811	GAUAUAC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
814	UCAGAUA	CUGAUGAGGCCGAAAGGCCCGAA	ACUAGGU
816	AGUCAGA	CUGAUGAGGCCGAAAGGCCCGAA	AUACUAG
818	AAAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUACU
824	AAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGA
825	GAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG
831	AAGUUGG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAA
832	GAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
838	AUAUUAG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGA
839	AAUAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGG
841	CUAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUU
844	CUUCUAA	CUGAUGAGGCCGAAAGGCCGAA	ALITIAGAA
846	uccuucu	CUGAUGAGGCCGAAAGGCCGAA	AUAIRIAG
847	AUCCUUC	CUGAUGAGGCCGAAAGGCCGAA	ANIJATITA
855	AGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCURE
858	UUGAGCA	CUGAUGAGGCCGAAAGGCCCGAA	AUTIDITION
859	GUUGAGC	CUGAUGAGGCCGAAAGGCCGAA	TITITIES
863	AGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACCABATI
869	ACCUCCA	CUGAUGAGGCCGAAAGGCCGAA	ACCITICA
877	UCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCIOCA
878	CUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACTIC
879	GCUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCTIC
880	GGCUCUG	CUGAUGAGGCCGAAAGGCCGAA	TOTALLA
889	GAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCOUNT
894	ACCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGIGAG
896	CAACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGTG
902	AUUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGG2
920	GGCAUUU	CUGAUGAGGCCGAAAGGCCGAA	ATHEMETER
921	UGGCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAITICTIT
930	UUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	TIACCOURT
942	CUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACTRETTS
943	UCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACTECTIC
944	AUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	7774231
952	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AIKTRISS
966	CAGCAUA	CUGAUGAGGCCGAAAGGCCGAA	ACCIPAG
968	AACAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACACCE
975	UGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACCATI
976	UUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCA
991	AUAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ATTICACT
992	CAUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAIMCAG
993	UCAUAUU	CUGAUGAGGCCGAAAGGCCGAA	ANIIGA
997	GUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	ALEGINA
1016	ACACAUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUG
1017	GACACAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCTITE
1024		CUGAUGAGGCCGAAAGGCCGAA	
1026	ACTUGAU	CUGAUGAGGCCGAA	ACACACE
1029	CAUACUII	CUGAUGAGGCCGAAAGGCCGAA	AUCACACA
1034	AUGUCCA	CUGAUGAGGCCGAAAGGCCGAA	ACTES SALES
			ACOUGAU

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1042	ACUCUUA CUGAUGAGGCCGAAAGGCCGAA AUGUCCA
1043	CACUCUU CUGAUGAGGCCGAAAGGCCGAA AAUGUCC
1044	UCACUCU CUGAUGAGGCCGAAAGGCCGAA AAAUGUC
1054	AAGGUCU CUGAUGAGGCCGAAAGGCCGAA AUUCACU
1061	CCAGUUG CUGAUGAGGCCGAAAGGCCGAA AGGUCUG
1062	UCCAGUU CUGAUGAGGCCGAAAGGCCGAA AAGGUCU
1072	UUGGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCCAG
1090	UCAGGAA CUGAUGAGGCCGAAAGGCCGAA AUGCUCU
1091	AUCAGGA CUGAUGAGGCCGAAAGGCCGAA AAUGCUC
1092	UAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAAUGCU
1093	UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAAAUGC
1099	AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCAGGA
1107	AGGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
1112	GGCCCAG CUGAUGAGGCCGAAAGGCCGAA AUGGGAG
1122	UUAAGGU CUGAUGAGGCCGAAAGGCCGAA AUGGCCC
1123	AUUAAGG CUGAUGAGGCCGAAAGGCCGAA AAUGGCC
1127	UGAGAUU CUGAUGAGGCCGAAAGGCCGAA AGGUAAU
1128	CUGAGAU CUGAUGAGGCCGAAAAGGCCGAA AAGGUAA
1131	UUACUGA CUGAUGAGGCCGAAAGGCCGAA AUUAAGG
1133	AUUUACU CUGAUGAGGCCGAAAGGCCGAA AGAUUAA
1137	UUCCAUU CUGAUGAGGCCGAAAGGCCGAA ACUGAGA
1146	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
1147	AUCACAA CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
1148	UAUCACA CUGAUGAGGCCGAAAGGCCGAA AAAUUCC
1149	AUAUCAC CUGAUGAGGCCGAAAGGCCGAA AAAAUUC
1155	GGCAGCA CUGAUGAGGCCGAAAGGCCGAA AUCACAA
1169	AAAGCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCAG
1175	UGGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGUA
1176	UUGGGGC CUGAUGAGGCCGAAAGGCCGAA AAGCAGU
1214	CCUUCUC CUGAUGAGGCCGAAAGGCCGAA AUCUCUC
1230	CAGGGGG CUGAUGAGGCCGAAAGGCCGAA ACACUUU
1239	ACUGUUA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
1241	ACACUGU CUGAUGAGGCCGAAAGGCCGAA AUACAGG
1249	UUCUGCG CUGAUGAGGCCGAAAGGCCGAA ACACUGU
1275	ACCUUCA CUGAUGAGGCCGAAAGGCCGAA AUCUUUU
1283	CGGAGGC CUGAUGAGGCCGAAAGGCCGAA ACCUUCA
1288	GAUGACG CUGAUGAGGCCGAAAGGCCGAA ACCCUAC
1292	AAGAGAU CUGAUGAGGCCGAAAGGCCGAA ACGGAGG
1295	CAGAAGA CUGAUGAGGCCGAAAGGCCGAA AUGACGG
1297	CCCAGAA CUGAUGAGGCCGAAAGGCCGAA AGAUGAC
1299	AUCCCAG CUGAUGAGGCCGAAAGGCCGAA AGAGAUG
1300	UAUCCCA CUGAUGAGGCCGAAAGGCCGAA AAGAGAU
1307	AUCCAUG CUGAUGAGGCCGAAAGGCCGAA AUCCCAG
L315	UCCCCAC CUGAUGAGGCCGAAAGGCCGAA AUCCAUG
1324	GCCUCAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCA
L334	AGGGAAG CUGAUGAGGCCGAAAGGCCGAA AUGCCUC
L335	AAGGGAA CUGAUGAGGCCGAAAGGCCGAA AAUGCCU
1337	UUAAGGG CUGAUGAGGCCGAAAGGCCGAA AGAAUGC
.338	GUUAAGG CUGAUGAGGCCGAAAGGCCGAA AAGAAUG

1342	AUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGGGAAG
1343	AAUUUGU CUGAUGAGGCCGAAAGGCCGAA .AAGGGAA
1350	CAGCUUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUU
1351	ACAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUUUGU
1352	AACAGCU CUGAUGAGGCCGAAAGGCCGAA AAAUUUG
1359	UGGGUAA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
1360	GUGGGUA CUGAUGAGGCCGAAAGGCCGAA AACAGCU
1361	AGUGGGU CUGAUGAGGCCGAAAAGGCCGAA AAACAGC
1362	UAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAAACAG
1369	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGGU
1373	AGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
1378	UUUUAAG CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
1379	UUUUUAA CUGAUGAGGCCGAAAGGCCGAA AAGGUGAG
1381	GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGGU
1382	AGGUUUU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
1390	UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
1392	AAUCUGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1393	UAAUCUG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1394	UUAAUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAGG
1399	UCAGCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
1400	UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AAUCUGAA
1412	AUCUUGU CUGAUGAGGCCGAAAGGCCCGAA ACUGUUC
1413	CAUCUUG CUGAUGAGGCCGAAAGGCCCGAA AACUGUU
1429	GGAGAGG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1433	GAAAGGA CUGAUGAGGCCGAAAAGGCCGAA AGGGAUG
1435	GAGAAAG CUGAUGAGGCCGAAAAGGCCGAA AGAGGGA
1438	GGGGAGA CUGAUGAGGCCGAAAAGGCCGAA AGGAGAG
1439	UGGGGAG CUGAUGAGGCCGAAAAGGCCGAA AAGGAGA
1440	AUGGGGA CUGAUGAGGCCGAAAAGCCCGAA AAAGGAG
1442	AUAUGG CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
1448	AAUUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
1455	UUAAGCA CUGAUGAGGCCGAAAGGCCGAA AUUGCAU
1456	AUUAAGC CUGAUGAGGCCGAAAGGCCGAA AAUUGCA
1460	UUACAUU CUGAUGAGGCCGAAAGGCCGAA AGCAAAU
1461	GUUACAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
1466	AAGAGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAA
1471	AAAAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUAC
1473	GCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1474	GGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
1476	AUGGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
1477	CAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAAGA
1478	ACAUGGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
1486	GAAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUGGC
L487	AGAAUGG CUGAUGAGGCCGAAAGGCCGAA AACAUGG
L488	CAGAAUG CUGAUGAGGCCGAAAAGGCCGAA AAACAUG
L492	AUGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
1493	GALICCCA CICALICACCASA AUGGAAA
500	GAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
.502	AAUUCAA CUGAUGAGGCCGAAAGGCCGAA AUGGCAG
	ACAAUUC CUGAUGAGGCCGAAAGGCCGAA AGAUGGC

WO 96/18736	
	176
1507	ACAAGAC CUGAUGAGGCCGAAAGGCCGAA AUUCAAG
1510	CUGACAA CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
1512	GGCUGAC CUGAUGAGGCCGAAAGGCCGAA AGACAAU
1515	AUUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAAGAC
1523	GAUAAUG CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
1524	AGAUAAU CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
1527	AAUAGAU CUGAUGAGGCCGAAAGGCCGAA AUGAAUU
. 1528	UAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAUGAAU
1530	UUUAAUA CUGAUGAGGCCGAAAGGCCGAA AUAAUGA
1532	UGUUUAA CUGAUGAGGCCGAAAGGCCGAA AGAUAAU
1534	AGUGUUU CUGAUGAGGCCGAAAGGCCCGAA AITAGAITA

UAGUGUU CUGAUGAGGCCGAAAGGCCGAA AAUAGAU

CUCAAAU CUGAUGAGGCCGAAAGGCCGAA AGUGUUU

1535

1542

PCT/US95/15516

Table BIV: Mouse B7-1 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	GaGUuUU a UACcUcA	108	Caucuuu a GCAucug
10	guuuau a ccucaau	108	CAUCUUU a gcaUCUG
10	GULUUAU a ccuCAAU	131	aUGCCAU C caGgcUU
14	UAUACCU c aAUAGAC	142	gCUuCUU U uUCuaCA
18	CcucAAU A gaCUCUu	142	acrincin a nacracy
18	CCUCaaU a gaCUCUU	143	CUUCUUU u UCUaCAU
18	CcUcAAU a GaCUcuU	143	CuUcUuU u uCuAcAU
23	AuaGaCU c uUACuaG	143	CUUCUUU U uCuAcaU
25	AGACUCU U aCUAGUU	143	CUUCUUU u UCUACAU
26	GACUCUU a CUAGUUU	144	UuCuUuU U cUaCAuC
29	UCUUACU a GuuUCuc	144	Uncumu a chacanc
29	UcUuACU a gUuuCuC	144	· UUCuuUU u cuaCAUC
29	UCUUaCU a guUUCUc	147	uUUUuCU a cAuCUCU
29	UCULLACU A GUUUCUC	153	HACAUCU C LIGUTUCU
34	CUaGUuU c UCUuuuU	165	UCUCGAU U UUUGUGA
34	CUAGUUU c UCUUUUU	165	uCUcgAU u UuuGUgA
34	cUAgUuU c uCuUuUU	165	ucucgAU U UUUGUGA
40	ucuCUuU U UCAGgUU	166.	CUCGAUU U uUgUgAG
41	cUCUuUU u caGGuUg	167	uCgAUuU u UGUGaGc
41	cuCUuUU U CAGgUUg	167	ucGauUU U UGUgAgC
42	ucuutuu c Agguugu	167	UCGAUUU u UgUgAGC
56	UGAAACU c AAcCuuC	168	cGAUUuU u gUgAGCC
56	UGAAACU C aACCUUC	168	cgAUUUU U GUGAgcc
62	uCAACCU U caaAGAC	197	GCUCCAU u GGCUCUA
62	UCAACCU U CAAAGAC	202	aUUGGCU c UagaUUc
62	UCAACCU u caaAGac	208	UCUAGAD U CCUGGCU
63	CAACCUU c aaAGACa	216	CCUGGCU u UcCcCau
73	aGAcAcU c UGuUCcA	217	cUGGCUU U CcCcaUc
77	acucugu u ccauuuc	217	CUGGCUU u CCCCAUC
78	CucUGUU c CauUUCU	217	CUGGCUU u CCcCauC
83	Uuccauu u cugugga	218	UGGCUUU c ccCaUCA
93	GUggacu a Allaggal	218	UGGCUUU C cCcaUca
93	gUgGacU a AUAGgaU	218	UGgCuUU c cCcaUCA
93	gUGgAcU a AMAGGAU	218	ugGcUUU c CCCAucA
96	GACUAAU a GGAUCAU	224	UCCCCAU c aUGuUCu
96 101	gacuAAU a gGAuCaU	224	UCCCCAU c aUGuucU
101	AUaGGAU c aUCuUuA	230	UCALIGUU C UCCAAAg
104	GGAUCAU C UUUAgCa	232	AUGUUCU C CAAAGCA
	GGAUCAU C UUUagcA	232	AUGUUCU c caaAGCA
106	AUCAUCU U UagcAUC	232	AugUUCU c cAAAgCa
107 107	UCAUCUU u AGCAUCU	241	AAAGCAU c UgAAGcu
107	uCaUCUU u AgcAuCU	241	aAAGCAU C UGAAGCu

241	AAAgcau C UGAAGcu	556	100.101
249	UGAAgeU A UGGCuuG	556	ACCUACU C UCUUAUC ACCUACU C UCUUAUC
264	CAAuUgU c AGuUGaU	560	ACUCUCU U aUCAUCC
287	CAcCaCU c CUcaagU	561	CUCUCUU a UCAUCCU
295	CUCaAgU u UCcaUGU	561	
295	CUCAAGU U UCCAUgu	561	CUCUCUU a UCAUCCU
296	uCAAgUU u ccAUgUc	566	CUCUCUU a UCauccu
297	CAAGUUU C CAUguCc	566	UUaUcAU C CUGGgcC
297	CAAGUUU c cAUGUCC	581	uUauCAU C CUGGGCC
314	GGCUcaU u cUUCUCu	583	UGGUCCU U UCAGACC
314	GgcuCAU U CUUCUCU	583	gucCUUU C AgaCcGG
315	GCuCAUU c UuCUcuU	598	Guccuuu c Agaccgg
315	acreynn c nncrenn		GGCACAU A CagcUGU
317	uCAUUCU U CuCUUug	608	gcUGUGU c GUUCaaA
318	CAUUCUU C ucuuugu	611	GUGUCGU u CAaaaGA
318	CAUUCUU C UCUUUgu	611	GUGUCGU U CaaAAGa
320	nncana c annada	612	UGUCGUU C aaAAGaA
320	Ancinca C nargaec	641	aUGaAGU u aaACaCU
322	CHICACA A REACCAR	649	AAAcacU U GGCUUUa
322	Chrarch a nancana	649	AaaCACU U gGCUUUA
323	UUCUCUU u gUGCUGC	655	UUggcuU u AGUAAAg
336		656	UGgcUUU a GUAAAgu
341	gcUGAUU c GUCUUUc	659	CultuaGU A AAGUugu
341	uUCGuCU u UCacAAG	664	GUaAaGU U gUCcaUC
342	UUCgucU u Ucacaag	667	AAGUUGU C CAUCAAA
343	UcGUCUU U CaCAagU	671 ·	UgUCcau C AAAGCUG
343	CGUCULU C ACAAGUG	682	gCUgAcU u CuCuACC
352	cGuCuUU c AcaAGUG	682	GCUGACU U CLCUACC
355	caAGUGU C uuCAGAu	682	GCUGacU U cuCuACc
382	gUgUcUU C AGaUGUU	683	CUGACUU C UCUACCC
408	UCcaAGU c AgUGaAA	683	CUGACUU c ucuAccC
414	gCUGCcU U GCCguuA	685	gACTUCT c VaCCCCc
414	UUGCCGU U aCAACUC	685	gaCUucU c UACCCcC
421	UUgCCgU u ACAAcUc	687	CUUCUCU A CCCCCAa
426	UaCAAcU c uCcUcAU	698	ccAACAU a ACUGagu
	CUCUCCU c aUgAAgA	698	CCaacAU A ACuGaGU
439	GaUGAgU c UGAaGaC	718	AACCCAU C UGCAGAC
452	acogaau c vacuoge	718	aacccAU c UGCAgac
454	CGAAUCU A CUGGCAA	729	AGACacu A AaAgGAu
484	GuGCUgU c UGucaUU	729	agAcAcU A aAAGGAU
484	GridCACA C AGriCYTA	729	agACAcU a AaAgGAU
488	ugueugu c auugeug	737	aAAGGAU u AccUGCU
503	gGAAacu A aAAGugu	737	aAAGGAU U ACCUGCu
503	ggAAAcU a AAagUGU	737	aaagGAU u ACCUGCU
520	CCCGAGU A UAAGAAC	745	accused u uscuuce
535 536	cGGAcUU U aUaUGAc	745	accUGcU u UGCUuCC
536	GGACUUU a UaUGACA	759	cGggGgU U uCCCAAA
538	AcUluUAU a UGACaac	759	
553	acuACCU a cUCUcUU	759	cGgGGGU u UcCcAaa
553	AcUaCcU a cUCUcUU	760	CGGGGGU U UCCCAAA
		, 55	GGGGGUU u CCCAAAG

760	gGGgGUU u cCCAaag		
760	GGGGGUU U CCCAAAG	1060	aAAUgcu u cUGUaAG
761	GGGGUUU C CCAAAGC	1060	AAAugCU u cUgUaAG
771	ayydccn c ccyyntc	1061	AAUGCUU C UGUaagc
771	Anacour C GCIOCOC	1080	AagcugU u UCAGAAG
776	ABAGCCU C GCUUCUC	1080	AAGCUGU U UCAgaag
776	CUCGCUU C UcUUggu	1081	AgCuGUU u CAgaAga
778	CUCGCUU C UCUUGGU	1121	acAGcCU U ACCuUcg
784	CACARCA C MACCARA	1121	AcAgccu u accudeg
803	UCUUGGU U GGAAAAU	1121	ACECCO U. ACCUUCG
803	GAGAAUU A CCUGGCA	1122	ACagccu u Accuucg
803	gagaauu a ccuggca	1126	CaGcCuU a cCUUCgG
812	gaghauu a ccuggch	1127	CUUACCU u CgGgccU
812	CUGGCAU C AAUACGA	1127	UUaCcUU c ggGcCUG
	CUGGCAU c aAuaCgA	1144	Unaccuu c GggCCUg
816	CAUCAAU A CGACAAU	1144	GaagCAU U AgCUgAA
816	caucaau a cgacaau	1145	gaAGcaU u AGCUGAA
824	CGACAAU U UCCCAGG	1160	aAgcAUU a GCUGAAC
825	gacaauu u cccagga	1162	AGACCGU C UUCCUUU
826	ACAAUUU C CCAGGAU	1163	Accoucu u Ccuuag
834	CCAGGAU C CUGAAUC	1163	CCGUCUU C CUUUAGU
841	CcUGaaU C ugAAUUG		CUUCCUU u AGUUCUU
841	ccugaau c ugaauug	1177	UUCUUCU c UguCCAU
850	gaauugu a caccauu	1181	UCuCugU C CAuGUGa
869	gcchacu a galluuca	1181	ucucugu c Cauguga
869	GCCAaCU a GAUUUca	1192	gUGGGAU A CAUGGUA
869	GCCAACU a gaUuUCa	1199	aCaUGGU a UUAugUG
873	acUaGAU u UCAaUAc	1201	AuGgUaU u aUGUGGc
873	ACUAGAU U UCAAUAC	1210	ugUGGcU C aUGaGGu
874	CUAGAUU U CAAUACG	1210	UGUGGCU C AUGAGGU
875	Uagauuu c aauacga	1223	GUACAAU C UUUCUUu
885	UACGACU C GCAACCA	1225	ACAAUCU U UCUUUCa
899	ACACCALL & GCAACCA	1225	ACAAUCU U UCUUUCA
899	ACACCAU u aAgUgUC	1226	CAAUCUU U CUUUCAG
906	ACACCAU u AAGUGUC	1227	aAucuuu c uuucagc
906	UaaGUGU c UcaUuAA	1227	AAucuuU C UUUCAGc
908	UAAGUGU C UCAULAA	1227	AAUCUUU c uUUcaGC
911	aGUGUCU C AUUAAAU	1229	UCUUUCU U UCAGCaC
916	GUCUCAU u AAAUAUG	1230	CUUUCUU U CAGCaCc
916	AUuAaaU a UGGaGAu	1252	cUgAUCU u UcggACA
943	AULAAAU A UGGAGAU	1274	acaAGAU a gAGuUaA
944	gAGgaCU U CACCUGG	1310	UGAgGaU u uCuUúCc
1001	AGGaCUU C Accugg	1312	aGgAUUU c UuUcCAu
1034	UGCUcUU u GggGCAg	1314	GAUUUCU :: JcCAuCA
1037	CAGUCGU c gUCauCG	1316	UUUCUUU c CAUCAGG
1043	UcGUCGU C AuCguUG	1320	UUUCCAU C AGGAAGC
1046	uCAUCGU U GUCAUCA	1320	ITTIO
1048	UCGUUGU C AUCAUCA	1339	UUUCcaU c aggaAGC
1060	uUguCaU c AuCAAAU	1355	GgCAagU u UgCUGGG
1060	aAAUGcU U CUGUaag	1437	CULUGAU C GCUUGAU
1000	AAaUgCU u cUgUaAG	1437	gUGguaU A aGAAAAA
			gugguau a Agaaaa

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1 475	
1475	gccuagu c uuacugc
1477	CUaGUCU U ACUgcaa
1487	ugCAaCU U gAUaUGU
1491	ACUUGAU a UGUCAUg
1491	aCUUgaU a UGuCAUG
1505	gUUUGgU U ggUGUcu
1530	uGCCcUU u uCUgAAg
1531	GCccUUU u CUGAagA
1532	CCCuUuU C UGAAGAg
1532	CcCuuuU C UGAaGAG
1644	CUaUGGU u gggAUGU
1652	ggGAuGU a AmAAcGG
1652	GgGAugU a aAaAcGG
1670	aUaAUAU a AaUAuUA
1674	uAuAAAU a UuAaaUa
1676	UaAaUAU u aAaUAAA
1677	AAauAUU a AAuaAAA
1677	AaaUAUU A AAuAaaA
1694	AGagUaU u gAGcAAA

Table BV: Mouse B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
8	UGAGGUA CUGAUGAGGCCGAAAGGCCGAA AAAACUC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
14	GUCUAUU CUGAUGAGGCCGAAAGGCCGAA AGGUAUA
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
23	CUAGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUAU
25	AACUAGU CUGAUGAGGCCGAAAGGCCGAA AGAGUCU
26	AAACUAG CUGAUGAGGCCGAAAGGCCGAA AAGAGUC
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
40	AACCUGA CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
42	ACAACCU CUGAUGAGGCCGAAAAGGCCGAA AAAAAGA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
56 62	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
62 62	GUCUUUG CUGAUGAGGCCGAAAGGCCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
63	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
73	UGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
73 77	UGGAACA CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
78	GAAAUGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGU
83	AGAAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
93	UCCACAG CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
96	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
101	IIAAGAII CICAICACCCCAAACCCCGAA AUUAGCC
104	UAAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCUAU
104	UGCUAAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
106	UGCUAAA CUGAUGAGGCCGAAAAGGCCGAA AUGAUCC
	GAUGCUA CUGAUGAGGCCGAAAGGCCCGAA AGAUGAU

107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
131	AAGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAU
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
147 .	AGAGAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
153	AGAAACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
166	CUCACAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCGAG
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCGA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCGA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCGA
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCG
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCCGAA	AAAAUCG
197	UAGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGC
202	GAAUCUA	CUGAUC SECCGAAAGGCCGAA	AGCCAAU
208	AGCCAGG	CUGAU: .GGCCGAAAGGCCGAA	AUCUAGA
216	AUGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCCGAA	AUGGGGA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
230	CUUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGA
232	UCCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232	UGCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232		CUGAUGAGGCCGAAAGGCCGAA	
241		·CUGAUGAGGCCGAAAGGCCCGAA	
241		CUGAUGAGGCCGAAAGGCCCGAA	
241	AGCUUCA	CUGAUGAGGCCGAAAGGCCCGAA	AUGCUUU
249	CAAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCA:
264	AUCAACU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
287		CUGAUGAGGCCGAAAGGCCGAA	
295		CUGAUGAGGCCGAAAGGCCGAA	

295	ACAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUUGAG
296	GACAUGG CUGAUGAGGCCGAAAAGGCCGAA AACUUGA
297	GGACAUG CUGAUGAGGCCGAAAAGGCCGAA AAACUUG
297	GGACAUG CUGAUGAGGCCGAAAAGGCCGAA AAACUUG
314	AGAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGAGCC
314	AGAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGAGCC
315	AAGAGAA GIRAKAAAGACCAAA AUGAGCC
315	AAGAGAA CUGAUGAGGCCGAAAGGCCGAA AAUGAGC AAGAGAA CUGAUGAGGCCGAAAGGCCGAA AAUGAGC
317	Charles Contractors and Augage
318	CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AGAAUGA ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGAAUG
318	ACAAGA CICAIDACCCGAAAGGCCGAA AAGAAGG
320	ACAAAGA CUGAUGAGGCCGAAAAGGCCGAA AAGAAUG
320	GCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
322	GCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA CAGCACA CUGAUGAGGCCGAAAGGCCCGAA AGAGAAG
322	CACCACA CIRALES ACCOCANA ACAGAAG
323	CAGCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAG
336	GCAGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
341	GAAAGAC CUGAUGAGGCCGAAAAGGCCGAA AAUCAGC
341	CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AGACGAA CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AGACGAA
342	ACUUGUG CUGAUGAGGCCGAAAGGCCGAA AAGACGA
343	CACUUGU CUGAUGAGGCCGAAAGGCCGAA AAAGACGA
343	CACUUGU CUGAUGAGGCCGAAAGGCCGAA AAAGACG
352	AUCUGAA CUGAUGAGGCCGAAAGGCCGAA ACACUUG
355	AACAUCU CUGAUGAGGCCGAAAGGCCGAA AAGACAC
382	UUUCACU CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
408	UAACGGC CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
414	GAGUUGU CUGAUGAGGCCGAAAGGCCGAA ACGCAA
414	GAGUUGU CUGAUGAGGCCGAAAGGCCGAA ACGGCAA
421	AUGAGGA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
426	UCUUCAU CUGAUGAGGCCGAAAGGCCGAA AGGAGAG
439	GUCUUCA CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
452	GCCAGUA CUGAUGAGGCCGAAAGGCCGAA AUUCGGU
454	UUGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAUUCG
484	AAUGACA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
484	AAUGACA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
488	CAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
503	ACACUUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCC
503	ACACUUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCC
520	GUUCUUA CUGAUGAGGCCGAAAGGCCGAA ACUCGGG
535	GUCAUAU CUGAUGAGGCCGAAAAGGCCGAA AAGUCCG
536	UGUCAUA CUGAUGAGGCCGAAAAGGCCGAA AAAGUCC
538	GUUGUCA CUGAUGAGGCCGAAAGGCCGAA AUAAAGU
553	AAGAGAG CUGAUGAGGCCGAAAAGGCCGAA AGGUAGU
553	AAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
56	GAUAAGA CUGAUGAGGCCGAAAGGCCGAA AGUAGTT
556	GAUAAGA CUGAUGAGGCCGAAAGGCCGAA AGUAGGU
60	GGAUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGU
61	AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
61	AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
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561	AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
566	GGCCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAUAA
566	GGCCCAG CUGAUGAGGCCGAAAGGCCCGAA AUGAUAA
581	GGUCUGA CUGAUGAGGCCGAAAGGCCGAA AGGACCA
583	CCGGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
583	CCGGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
598	ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AUGUGCC
608	UUUGAAC CUGAUGAGGCCGAAAGGCCGAA ACACAGC
611	UCUUUUG CUGAUGAGGCCGAAAGGCCGAA ACGACAC
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649	UAAAGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
649	UAAAGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
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659	ACAACUU CUGAUGAGGCCGAAAGGCCGAA ACUAAAG
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671	CAGCUUU CUGAUGAGGCCGAAAGGCCGAA AUGGACA
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729 737	AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
	AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
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737 745	AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
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745 759	GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
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760	CUUUGGG CUGAUGAGGCCGAAAAGGCCGAA AACCCCC
761	CUUUGGG CUGAUGAGGCCGAAAAGGCCGAA AACCCCC
761 771	GCUUUGG CUGAUGAGGCCGAAAGGCCGAA AAACCCC
771	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUUU
• • •	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUUU

776	ACCAAGA CUGAUGAGGCCGAAAGGCCGAA AAGCGAG
776	ACCAAGA CUGAUGAGGCCGAAAGGCCGAA AAGCGAG
778	CAACCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGCG
784	AUUUUCC CUGAUGAGGCCGAAAGGCCGAA ACCAAGA
803	UGCCAGG CUGAUGAGGCCGAAAGGCCGAA AAUUCUC
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812	UCGUAUU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
816	AUUGUCG CUGAUGAGGCCGAAAGGCCGAA AUUGAUG
816	AUUGUCG CUGAUGAGGCCGAAAGGCCGAA AUUGAUG
824	CCUGGGA CUGAUGAGGCCGAAAGGCCGAA AUUGUCG
825	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA AAUUGUC
826	AUCCUGG CUGAUGAGGCCGAAAGGCCGAA AAAUUGU
834	GAUUCAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGG
841	CAAUUCA CUGAUGAGGCCGAAAGGCCGAA AUUCAGG
841	CAAUUCA CUGAUGAGGCCGAAAGGCCGAA AUUCAGG
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869	UGAAAUC CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
869	UGAAAUC CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
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874	CGUAUUG CUGAUGAGGCCGAAAGGCCGAA AAUCUAG
875	UCGUAUU CUGAUGAGGCCGAAAGGCCGAA AAAUCUA
885	UGGUUGC CUGAUGAGGCCGAAAGGCCGAA AGUCGUA
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899	GACACUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
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911	CAUAUUU CUGAUGAGGCCGAAAGGCCGAA AUGAGAC
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1049	AUUUGAU CUGAUGAGGCCGAAAGGCCGAA AUGACAA
1060	CUUACAG CUGAUGAGGCCGAAAGGCCGAA AGCAUUU
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1061	GCUUACA CUGAUGAGGCCGAAAGGCCGAA AAGCAUU
1080	CUUCUGA CUGAUGAGGCCGAAAAGGCCGAA ACAGCTU
1080	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACAGCTT
_	Sour Consumerce MANGELLIAA ACAGCUS

1081	UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AACAGCU
1121	CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
1121	CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
1121	CGAAGGU CUGAIGAGGCCGAAAGGCCGAA AGGCUGU
1122	COGAAGG CUGAUGAGGCCGAAAAGGCCGAA AAGGCUG
1126	AGGCCCG CUGAUGAGGCCGAAAGGCCGAA AGGUAAG
1127	CAGGCCC CUGAUGAGGCCGAAAGGCCGAA AAGGUAAG
1127	CAGGCCC CUGAUGAGGCCGAAAGGCCGAA AAGGUAA
1144	UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC
1144	UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC
1145	GUUCAGC CUGAUGAGGCCGAAAGGCCGAA AAUGCUUC
1160	AAAGGAA CUGAUGAGGCCGAAAGGCCGAA ACGGUCU
1162	CUAAAGG CUGAUGAGGCCGAAAGGCCGAA AGACGGU
1163	ACUANAG CUGAUGAGGCCGANAGGCCGAN AGACGG
1167	AAGAACU CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
1177	AUGGACA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
1181	CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA
1181	CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA
1192	UACCAUG CUGAUGAGGCCGAAAGGCCGAA AUCCCAC
1199	CACAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUGU
1201	GCCACAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
1210	ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA
1210	ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA
1223	AAAGAAA CUGAUGAGGCCGAAAAGGCCGAA AUUGUAC
1225	UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
1225	UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUTU
1226	CUGAAAG CUGAUGAGGCCGAAAAGGCCGAA AAGA
1227	GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGU
1227	GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
1227	GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
1229	GUGCUGA CUGAUGAGGCCGAAAAGGCCGAA AGAAAGA
1230	GGUGCUG CUGAUGAGGCCGAAAGGCCGAA AAGAAAG
1252	UGUCCGA CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
1274	UUAACUC CUGAUGAGGCCGAAAGGCCGAA AUCUUGU
1310	GGAAAGA CUGAUGAGGCCGAAAGGCCCGAA AUCCUCA
1312	AUGGAAA CUGAUGAGGCCGAAAAGGCCGAA AAAUCCU
1314	UGAUGGA CUGAUGAGGCCGAAAGGCCGAA AGAAAUC
1316	CCUGAUG CUGAUGAGGCCGAAAGGCCGAA AAAGAAA
1320	GCUUCCU CUGAUGAGGCCGAAAGGCCGAA AIRCCAAA
1320	GCUUCCU CUGAUGAGGCCGAAAGGCCGAA AIRCCAAA
1339	CCCAGCA CUGAUGAGGCCGAAAGGCCGAA ACTITISCC
1355	AUCAAGC CUGAUGAGGCCGAAAGGCCGAA AUCAALG
1437	UUUUUCU CUGAUGAGGCCGAAAGGCCGAA AIIACCAC
1437	UUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUACCAC
1475	GCAGUAA CUGAUGAGGCCGAAAGGCCGAA ACUAGGC
1477	UUGCAGU CUGAUGAGGCCGAAAGGCCGAA AGACTIAG
1487	ACAUAUC CUGAUGAGGCCGAAAGGCCGAA AGUUCCA
1491	CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGI
1491	CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGU

WO 96/18736	187
1505	AGACACC CUGAUGAGGCCGAAAGGCCGAA ACCAAAC
1530	CUUCAGA CUGAUGAGGCCGAAAGGCCGAA AAGGGCA
. 1531	UCUUCAG CUGAUGAGGCCGAAAGGCCGAA AAAGGGC
1532	CUCUUCA CUGAUGAGGCCGAAAGGCCGAA AAAAGGG
1532	CUCUUCA CUGAUGAGGCCGAAAGGCCGAA AAAAGGG
1644	ACAUCCC CUGAUGAGGCCGAAAGGCCGAA ACCAUAG
1652	CCGUUUU CUGAUGAGGCCGAAAGGCCCGAA ACAUCCC
1652	CCGUUUU CUGAUGAGGCCGAAAGGCCGAA ACALICCC
1670	UAAUAUU CUGAUGAGGCCGAAAGGCCGAA AUAUUAU
1674	UAUUUAA CUGAUGAGGCCGAAAGGCCGAA AUUUAUA
1676	UUUAUUU CUGAUGAGGCCGAAAGGCCGAA AUALUUUA
1677	UUUUAUU CUGAUGAGGCCGAAAGGCCGAA AAUAUUU

UUUUAUU CUGAUGAGGCCGAAAGGCCGAA AAUAUUU

UUUGCUC CUGAUGAGGCCGAAAGGCCGAA AUACUCU

1677

1694

PCT/US95/15516

Table BVI: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
16	GAAAGCU U UGCUUCU	271	UAGUAGU A UUUUGGC
17	AAAGCUU U GCUUCUC	273 .	GUAGUAU U UUGGCAG
21	CUUUGCU U CUCUGCU	274	UAGUAUU U UGGCAG
22	UUUGCUU C UCUGCUG	275	AGUAUUU U GGCAGGA
24	ACCANCA C ACCACCA	294	GAAAACU U GGUUCUG
34	CUGCUGU A ACAGGGA	298	ACUUGGU U CUGAAUG
44	AGGGACU A GCACAGA	299	CUUGGUU C UGAAUGA
70	GUGGGGU C AUUUCCA	310	AUGAGGU A UACUUAG
73	GGGUCAU U UCCAGAU	312	GAGGUAU A CUUAGGC
74	GGUCAUU U CCAGAUA	315	GUAUACU U AGGCAAA
75	GUCAUUU C CAGAUAU	316	UAUACUU A GGCAAAG
81	UCCAGAU A UUAGGUC	330	GAGAAAU U UGACAGU
83	CAGAUAU U AGGUCAC	331	AGAAAUU U GACAGUG
84	AGAUAUU A GGUCACA	340	ACAGUGU U CAUUCCA
88	AUUAGGU C ACAGCAG	341	CAGUGUU C AUUCCAA
113	AAUGGAU C CCCAGUG	344	UGUUCAU U CCAAGUA
125	GUGCACU A UGGGACU	345	GUUCAUU C CAAGUAU
137	ACUGAGU A ACAUUCU	351	UCCAAGU A UAUGGGC
142	GUAACAU U CUCUUUG	353	CAAGUAU A UGGGCCG
143	UAACAUU C UCUUUGU	368	CACAAGU U UUG? - R;
145	ACAUUCU C UUUGUGA	369	ACAAGUU U UGAUUCG
147	AUUCUCU U UGUGAUG	370	CAAGUUU U GAUUCGG
148	UUCUCUU U GUGAUGG	374	UUUUGAU U CGGACAG
159	AUGGCCU U CCUGCUC	375	UUUGAUU C GGACAGU
160	neescan c caecaen	383	GGACAGU U GGACCCU
166	UCCUGCU C UCUGGUG	397	UGAGACU U CACAAUC
168	CUGCUCU C UGGUGCU	398	GAGACUU C ACAAUCU
179	UGCUGCU C CUCUGAA	404	UCACAAU C UUCAGAU
182	UGCUCCU C UGAAGAU	406	ACAAUCU U CAGAUCA
190	UGAAGAU U CAAGCUU	407	CAAUCUU C AGAUCAA
191	GAAGAUU C AAGCUUA	412	UUCAGAU C AAGGACA
197	UCAAGCU U AUUUCAA	426	AAGGGCU U GUAUCAA
. 198	CAAGCUU A UUUCAAU	429	GGCUUGU A UCAAUGU
200	AGCUUAU U UCAAUGA	431	CUUGUAU C AAUGUAU
201	GCUUAUU U CAAUGAG	437	UCAAUGU A UCAUCCA
202	CUUAUUU C AAUGAGA	439	AAUGUAU C AUCCAUC
231	UGCCAAU U UGCAAAC	442	GUAUCAU C CAUCACA
232	GCCAAUU U GCAAACU	446	CAUCCAU C ACAAAAA
240	GCAAACU C UCAAAAC	469	GAAUGAU U CGCAUCC
242	AAACUCU C AAAACCA	470	AAUGAUU C GCAUCCA
265	GUGAGCU A GUAGUAU	475	UUCGCAU C CACCAGA
268	AGCUAGU A GUAUUUU	488	GAUGAAU U CUGAACU

489	AUGAAUU C UGAACUG	721	OGUCUGU U UCAUUCC
498	GAACUGU C AGUGCUU	722	GUCUGUU U CAUUCCC
505	CAGUGCU U GCUAACU	723	UCUGUUU C AUUCCCU
509	GCUUGCU A ACUUCAG	726	GUUUCAU U CCCUGAU
513	GCUAACU U CAGUCAA	727	UUUCAUU C CCUGAUG
514	CUAACUU C AGUCAAC	736	CUGAUGU U ACGAGCA
518	CUUCAGU C AACCUGA	737	UGAUGUU A CGAGCAA
529	CUGAAAU A GUACCAA	746	GAGCAAU A UGACCAU
532	AAAUAGU A CCAAUUU	754	UGACCAU C UUCUGUA
538	UACCAAU U UCUAAUA	756	ACCAUCU U CUGUAUU
539	ACCAAUU U CUAAUAU	757	CCAUCUU C UGUAUUC
540	CCAAUUU C UAAUAUA	761	CUUCUGU A UUCUGGA
542	AAUUUCU A AUAUAAC	763	UCUGUAU U CUGGAAA
545	UUCUAAU A UAACAGA	764	CUGUAUU C UGGAAAC
547	CUAAUAU A ACAGAAA	787	CGCGGCU U UUAUCUU
561	AAUGUGU A CAUAAAU	788	GCGGCUU U UAUCUUC
565	UGUACAU A AAUUUGA	789	CGGCUUU U AUCUUCA
569	CAUAAAU U UGACCUG	790	GGCUUUU A UCUUCAC
570	AUAAAUU U GACCUGC	792	CUUUUAU C UUCACCU
579	ACCUGCU C AUCUAUA	794	UUUAUCU U CACCUUU
582	UGCUCAU C UAUACAC	795	UUAUCUU C ACCUUUC
584	CUCAUCU A UACACGG	800	UUCACCU U UCUCUAU
586	CAUCUAU A CACGGUU	801	UCACCUU U CUCUAUA
593	ACACGGU U ACCCAGA	802	CACCUUU C UCUAUAG
594	CACGGUU A CCCAGAA	804	CCUUUCU C UAUAGAG
605	AGAACCU A AGAAGAU	806	UUUCUCU A UAGAGCU
619	UGAGUGU U UUGCUAA	. 808	UCUCUAU A GAGCUUG
620	GAGUGUU U UGCUAAG	814	UAGAGCU U GAGGACC
621	AGUGUUU U GCUAAGA	824	GGACCCU C AGCCUCC
625	UUUUGCU A AGAACCA	830	UCAGCCU C CCCCAGA
638	CAAGAAU U CAACUAU	844	ACCACAU U CCUUGGA
639	AAGAAUU C AACUAUC	845	CCACAUU C CUUGGAU
644	UUCAACU A UCGAGUA	848	CAUUCCU U GGAUUAC
646	CAACUAU C GAGUAUG	853	
651	AUCGAGU A UGAUGGU	854	CUUGGAU U ACAGCUG
659	UGAUGGU A UUAUGCA	862	UUGGAUU A CAGCUGU
661	AUGGUAU U AUGCAGA	865	CAGCUGU A CUUCCAA
662	UGGUAUU A UGCAGAA	866	CUGUACU U CCAACAG
672	CAGAAAU C UCAAGAU	874	UGUACUU C CAACAGU
674	GAAAUCU C AAGAUAA	875	CAACAGU U AUUAUAU AACAGUU A UUAUAUG
680	UCAAGAU A AUGUCAC	877	
685	AUAAUGU C ACAGAAC		CAGUUAU U AUAUGUG
696	GAACUGU A CGACGUU	878	AGUUAUU A UAUGUGU
703	. ACGACGU U UCCAUCA	880	UUAUUAU A UGUGUGA
704	CGACGUU U CCAUCAG	892	UGAUGGU U UUCUGUC
705	GACGUUU C CAUCAGC	893	GAUGGUU U UCUGUCU
709		894	AUGGUUU U CUGUCUA
703 714	UUUCCAU C AGCUUGU	895	UGGUUUU C UGUCUAA
714 717	AUCAGCU U GUCUGUU	899	UUUCUGU C UAAUUCU
, 1 /	AGCUUGU C UGUUUCA	901	UCUGUCU A AUUCUAU

904	GUCUAAU U CUAUGGA
905	UCUAADU C UAUGGAA
907	UAAUUCU A UGGAAAU
935	GCGGCCU C GCAACUC
942	CGCAACU C UUAUAAA
944	CAACUCU U AUAAAUG
945	AACUCUU A UAAAUGU
947	CUCUUAU A AAUGUGG
1009	AAAAAAU C CAUAUAC
1013	AAUCCAU A UACCUGA
1015	UCCAUAU A CCUGAAA
1026 .	GAAAGAU C UGAUGAA
1045	AGOGUGU U UUUAAAA
1046	GCGUGUU U UUAAAAG
1047	CGUGUUU U UAAAAGU
1048	GUGUUUU U AAAAGUU
1049	UGUUUUU A AAAGUUC
1055	UAAAAGU U CGAAGAC
1056	AAAAGUU C GAAGACA
1065	AAGACAU C UUCAUGC
1067	GACAUCU U CAUGCGA
1068	ACAUCUU C AUGCGAC
1085	AAGUGAU A CAUGUUU
1091	UACAUGU U UUUAAUU
1092	ACAUGUU U UUAAUUA
1093	CAUGUUU U UAAUUAA
1094	AUGUUUU U AAUUAAA
1095	UGUUUUU A AUUAAAG
1098	UUUUAAU U AAAGAGU
1099	UUUAAUU A AAGAGUA

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Table BVII: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
16	AGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
17	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
21	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAAAG
22	CAGCAGA CUGAUGAGGCCGAAAAGGCCGAA AAGCAAA
24	AGCAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCA
34	UCCCUGU CUGAUGAGGCCGAAAGGCCGAA ACACCAG
44	UCUGUGC CUGAUGAGGCCGAAAGGCCGAA ACTICCCTI
70	UGGAAAU CUGAUGAGGCCGAAAGGCCCGAA ACCCCAC
73	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUGACCC
74	UAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUGACC
75	AUAUCUG CUGAUGAGGCCGAAAGGCCCGAA AAAIRGAC
81	GACCUAA CUGAUGAGGCCGAAAGGCCGAA AIRTICGA
83	GUGACCU CUGAUGAGGCCGAAAGGCCGAA AUAUCUC
84	UGUGACC CUGAUGAGGCCGAAAGGCCCGAA AATIATETT
88	CUGCUGU CUGAUGAGGCCGAAAGGCCGAA ACCTIAATI
113	CACUGGG CUGAUGAGGCCGAAAGGCCCGAA AIICCAITH
125	AGUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
137	AGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACUCACU
142	CAAAGAG CUGAUGAGGCCGAAAGGCCCC AUGUITAC
143	ACAAAGA CUGAUGAGGCCGAAAGGCCCGAA AAIRTIIIA
145	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAIXTI
147	CAUCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
148	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
159	GAGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
160	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCA
166	CACCAGA CUGAUGAGGCCGAAAGGCCGAA ACCACCA
168	AGCACCA CUGAUGAGGCCGAAAGGCCGAA AGAGCAG
179 182	UUCAGAG CUGAUGAGGCCGAAAGGCCGAA ACCACCA
192	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGGACCA
190 191	AAGCUUG CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
191	UAAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
198	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGA
200	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
201	UCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
202	CUCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
231	UCUCAUU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
232	GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
240	AGUUUGC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
242	GUUUUGA CUGAUGAGGCCGAAAGGCCGAA AGUUUGC
265	UGGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUU
403	AUACUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCAC

268	AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACUAGCU
271	GCCAAAA CUGAUGAGGCCGAAAGGCCGAA ACUACUA
273	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
274	CCUGCCA CUGAUGAGGCCGAAAGGCCCGAA AAUACUA
275	UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUACUA
294	CAGAACC CUGAUGAGGCCGAAAGGCCGAA AGUUUUC
298	CAUUCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAGU
299	UCAUUCA CUGAUGAGGCCGAAAGGCCGAA AACCAAG
310	CUAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUCAU
312	GCCUAAG CUGAUGAGGCCGAAAGGCCGAA AUACCUC
315	UUUGCCU CUGAUGAGGCCGAAAGGCCGAA AGUAUAC
316	CUUUGCC CUGAUGAGGCCGAAAGGCCGAA AAGUAUA
330	ACUGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUCUC
331	CACUGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUCU
340	UGGAAUG CUGAUGAGGCCGAAAGGCCGAA ACACUGU
341	UUGGAAU CUGAUGAGGCCGAAAGGCCGAA AACACUG
344	UACUUGG CUGAUGAGGCCGAAAGGCCGAA AUGAACA
345	AUACUUG CUGAUGAGGCCGAAAGGCCGAA AAUGAAC
351	GCCCAUA CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
353	CGGCCCA CUGAUGAGGCCGAAAGGCCGAA AUACUIUS
368	GAAUCAA CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
369	CGAAUCA CUGAUGAGGCCGAAAGGCCGAA AACUITTI
370	CCGAAUC CUGAUGAGGCCGAAAGGCCGAA AAACTIIK
374	CUGUCCG CUGAUGAGGCCGAAAGGCCGAA AUCAAAA
375	ACUGUCC CUGAUGAGGCCGAAAGGCCGAA AAUCAAA
383	AGGGUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUCC
397	GAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGTI IICA
398	AGAUUGU CUGAUGAGGCCGAAAGGCCCGAA AAVOCUC
404 406	AUCUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGUGA
407	UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
412	UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AAGAUUG
426	UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
429	UUGAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
431	ACAUUGA CUGAUGAGGCCGAAAGGCCGAA ACAAGCC
437	AUACAUU CUGAUGAGGCCGAAAGGCCGAA AUACAAG
439	UGGAUGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGA
442	GAUGGAU CUGAUGAGGCCGAAAGGCCGAA AUACAUU
446	UGUGAUG CUGAUGAGGCCGAAAGGCCGAA AUGAUAC
469	GGAUGCG CUGAUGAGGCCGAAAGGCCGAA AUGGAUC
470	UGGAUGC CUGAUGAGGCCGAAAGGCCGAA AUCAUU
475	UCUGGUG CUGAUGAGGCCGAAAGGCCGAA AUGCGAA
488	AGUUCAG CUGAUGAGGCCGAAAGGCCGAA AUUCALC
489	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA AAUUCAU
498	AAGCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
505	AGUUAGC CUGAUGAGGCCGAAAGGCCGAA AGCACUG
509	CUGAAGU CUGAUGAGGCCGAAAGGCCGAA AGCAAGC
513	UUGACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAGC
514	GUUGACU CUGAUGAGGCCGAAAAGGCCGAA AAGUUAG
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518	UCAGGUU CUGAUGAGGCCGAAAGGCCCGAA ACUGAAG
529	UUGGUAC CUGAUGAGGCCGAAAGGCCGAA AUUUCAC
532	AAAUUGG CUGAUGAGGCCGAAAGGCCCGAA ACUAUUU
538	UAUUAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGUA
539	AUAUUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
540	UAUAUUA CUGAUGAGGCCGAAAGGCCCGAA AAAUUGG
542	GUUAUAU CUGAUGAGGCCGAAAGGCCCGAA AGAAAUU
545	UCUGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
547	UUUCUGU CUGAUGAGGCCGAAAGGCCCGAA AUAUUAG
561	AUUUAUG CUGAUGAGGCCGAAAGGCCGAA ACACAUU
565	UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
569	CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
570	GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUAU
579	UAUAGAU CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
582	GUGUAUA CUGAUGAGGCCGAAAGGCCGAA AUGAGCA
584	CCGUGUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
586	AACOGUG CUGAUGAGGCCGAAAGGCCGAA AUAGAUG
593	UCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCGUGU
594	UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AACCGUG
605	AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
619	UUAGCAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
620	CUUAGCA CUGAUGAGGCCGAAAGGCCGAA AACACUC
621	UCUUAGC CUGAUGAGGCCGAAAGGCCGAA AAACACU
625	UGGUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
638	AUAGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUG
639	GAUAGUU CUGAUGAGGCCGAAAAGGCCGAA AAUUCUU
644	UACUCGA CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
646	CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUG
651	ACCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCGAU
659	UGCAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
661	UCUGCAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
662	UUCUGCA CUGAUGAGGCCGAAAAGGCCGAA AAUACCA
672	AUCUUGA CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
674	UUAUCUU CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
680	GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
685	GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAU
696	AACGUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
703	UGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACGUCGU
704	CUGAUGG CUGAUGAGGCCGAAAGGCCGAA AACGUCG
705	GCUGAUG CUGAUGAGGCCGAAAAGGCCGAA AAACGUC
709	ACAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
714	AACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
717	UGAAACA CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
721	GGAAUGA CUGAUGAGGCCGAAAGGCCGAA ACAGACA
722	GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAC
723	AGGGAAU CUGAUGAGGCCGAAAGGCCGAA AACAGA
726	AUCAGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAC
727	CAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
736	UGCUCGU CUGAUGAGGCCGAAAGGCCGAA ACAUCAG
	TO THE TOWN WOLL GAR ACAUCAG

737	UUGCUCG CUGAUGAGGCCGAAAGGCCCGAA AACAUCI
746	AUGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGCU
754	UACAGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCI
756	AAUACAG CUGAUGAGGCCGAAAGGCCGAA AGAUGG
757	GAAUACA CUGAUGAGGCCGAAAGGCCGAA AAGAUGC
761	UCCAGAA CUGAUGAGGCCGAAAGGCCGAA ACAGAAC
763	UUUCCAG CUGAUGAGGCCGAAAGGCCGAA AUACAGI
764	GUUUCCA CUGAUGAGGCCGAAAGGCCGAA AAUACAC
787	AAGAUAA CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
788	GAAGAUA CUGAUGAGGCCGAAAGGCCGAA AAGCCGC
789	UGAAGAU CUGAUGAGGCCGAAAGGCCGAA AAAGCCG
790	GUGAAGA CUGAUGAGGCCGAAAAGGCCGAA AAAAGCC
792	AGGUGAA CUGAUGAGGCCGAAAGGCCGAA AUAAAAG
794	AAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGAUAAA
795	GAAAGGU CUGAUGAGGCCGAAAAGGCCGAA AAGAUAA
800	AUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAA
801	UAUAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUGA
802	CUAUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGGUG
804	CUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
806	AGCUCUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
808	CAAGCUC CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
814	GGUCCUC CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
824	GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
830	UCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
844	UCCAAGG CUGAUGAGGCCGAAAGGCCGAA AUGUGGU
845	AUCCAAG CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
848	GUAAUCC CUGAUGAGGCCGAAAGGCCGAA AGGAAUG
853	CAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCCAAG
854	ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA
862	UUGGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCUG
865	CUGUUGG CUGAUGAGGCCGAAAGGCCGAA AGUACAG
866	ACUGUUG CUGAUGAGGCCGAAAGGCCGAA AAGUACA
874	AUAUAAU CUGAUGAGGCCGAAAGGCCGAA ACUGUUG
875	CAUAUAA CUGAUGAGGCCGAAAGGCCGAA AACUGUU
877	CACAUAU CUGAUGAGGCCGAAAGGCCGAA AUAACUG
878	ACACAUA CUGAUGAGGCCGAAAGGCCGAA AAUAACU
880	UCACACA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA
892	GACAGAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
893	AGACAGA CUGAUGAGGCCGAAAGGCCGAA AACCAUC
894	UAGACAG CUGAUGAGGCCGAAAGGCCGAA AAACCAU
895	UUAGACA CUGAUGAGGCCGAAAGGCCGAA AAAACCA
899	AGAAUUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
901	AUAGAAU CUGAUGAGGCCGAAAGGCCGAA AGACAGA
904	UCCAUAG CUGAUGAGGCCGAAAGGCCGAA AUUAGAC
905	UUCCAUA CUGAUGAGGCCGAAAGGCCGAA AAUUAGA
907	AUTUUCCA CUGAUGAGGCCGAAAGGCCGAA AGAAUUA
935	GAGUUGC CUGAUGAGGCCGAAAGGCCGAA AGGCCCCC
942	UUUAUAA CUGAUGAGGCCGAAAGGCCGAA AGUUGCG
944	CAUUUAU CUGAUGAGGCCGAAAGGCCGAA AGAGUUG

945	ACAUUUA CUGAUGAGGCCGAAAGGCCGAA AAGAGUU
947	CCACAUU CUGAUGAGGCCGAAAGGCCGAA AUAAGAG
1009	GUAUAUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUUU
1013	UCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUGGAUU
1015	UUUCAGG CUGAUGAGGCCGAAAGGCCGAA AUAUGGA
1026	UUCAUCA CUGAUGAGGCCGAAAGGCCGAA AUCUUUC
1045	UUUUAAA CUGAUGAGGCCGAAAGGCCGAA ACACGCU
1046	CUUUUAA CUGAUGAGGCCGAAAGGCCGAA AACACGC
1047	ACUUUUA CUGAUGAGGCCGAAAGGCCGAA AAACACG
1048	AACUUUU CUGAUGAGGCCGAAAGGCCGAA AAAACAC
1049 .	GAACUUU CUGAUGAGGCCGAAAGGCCGAA AAAAACA
1055	GUCUUCG CUGAUGAGGCCGAAAGGCCGAA ACUUUUA
1056	UGUCUUC CUGAUGAGGCCGAAAGGCCGAA AACUUUU
1065	GCAUGAA CUGAUGAGGCCGAAAGGCCGAA AUGUCUU
1067 .	UCGCAUG CUGAUGAGGCCGAAAGGCCGAA AGAUGUC
1068	GUCGCAU CUGAUGAGGCCGAAAGGCCGAA AAGAUGU
1085	AAACAUG CUGAUGAGGCCGAAAGGCCGAA AUCACUU
1091	AAUUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUGUA
1092	UAAUUAA CUGAUGAGGCCGAAAGGCCGAA AACAUGU
1093	UUAAUUA CUGAUGAGGCCGAAAGGCCGAA AAACAUG
1094	UUUAAUU CUGAUGAGGCCGAAAGGCCGAA AAAACAU
1095	CUUUAAU CUGAUGAGGCCGAAAGGCCGAA AAAAACA
1098	ACUCUUU CUGAUGAGGCCGAAAGGCCGAA AUUAAAA
1099	UACUCUU CUGAUGAGGCCGAAAGGCCGAA AAUUAAA

Table BVIII: Mouse B7-2 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
47	AcGGACU u GaACAac	194	cuUAuUU C aAUGGgA
47	aCggACU u gaAcAAC	208	acUGCaU a UCUGCcG
66	CUccugu a gacgugu	210	UGCaUaU C UGCcGug
66	CUCcUgU A gAcGUGu	223	UGCCCAU U UACAAAg
74	gAcGUGU u CcagAAc	223	UGCcCAU u UAcAaAg
83	CaGaACU U aCggaAG	224	GCCCAUU U aCAAAgg
134	caAuCcU U aUCUUUG	225	ccCAUUU a CAaAggc
134	CaauceU U AUCUUug	225	CccaUUU a cAAAgGc
134	caAUCcU u AuCUUUg	242	AAaACAU a agCcUGa
134	CAAUCCU U AUCUUUG	260	AGCUGGU A GUAUUUU
134	CAAUCCU U AUCUUUG	260	aGCuGgU a gUAUuUU
135	aAuCcUU a UCUUUGU	263	Ugguagu a uuuuggc
135	aAuCcUU a UCUuUgu	263	UGgUaGU a UUuUGgC
135	Aauccuu a ucuuugu	265	GUAGUAU U UUGGCAG
135	aAUccUU a UCUuUgU	265	guAGUAU u UuGGCaG
137	uccuuau c uuuguga	266	UAGUAUU U UGGCAGG
137	Uccuuau c uluguga	266	WAGUAUU U UGgcAgG
137	UCCUUAU c uuUGugA	266	UAgUauU u UGGcAgg
139	cUUaUCU U UGUGAca	267	AGUAUUU U GGCAGGA
140	UUaUCUU U GUGAcaG	267	AGUaUUU U GgcAgGA
140	UUaUcuU U guGACAG	286	CAAAAgU U GGUUCUG
149	UGAcaGU c UUGCUgA	286	CAAaagU U GgUUCuG
151	AcAGucU U GCUgaUC	290	AgUUGGI U CUGUACG
151	AcaGuCU U gCUGaUC	291	gUUGGUU C UGUACGA
. 158	UgCuGAU c UcAGaUg	295	GUUCugU a CgAGcAc
158	UgCUGaU C UCaGaUG	304	GAGCacU A uUUgGGC
158	UGcUgAU c uCAgaUg	307	CACUAUU u GGGCACA
158	UgCugAU c UCagAUg	323	AGAAACU U GALAGUG
160	CUGAUCU C aGaUGCU	343	gCCAAGJ A ccUGGGC
160	cUGaUcU c AgAuGcU	343	gCCAagU a CCUgGGc
170	AUGCUGU u UcCgUgG	361	ACGAGCU U UGACAGG
171	UGCUGuU u CcgUGgA	381	cUGGACU c UacGACU
172 189	gCUgUuU C cgUgCAG	383	GGACUCU A CGACUUC
189	GCAAGCU u AUUUCAA	383	GGACUCU a cGaCUUC
189	gCAAGCU U AUUUCAA	389	uAcGacU u CaCAaUG
190	GCAAGCUU AUUUCAA CAAGCUU A UUUCAAU	389	UacGACU U CACAAUg
190		390	acGACUT C ACAAUgU
192	CaAgcUU a uUUcaAU	390	ACGACUT c acAAUgU
192	AGCUUAU U UCAAUGg	398	ACAaUGU U CAgauCA
192	agcuuau u ucaaugg	398	ACAAUGU U CAGAUCA
193	GCUUAUU U CAAUGGG	398	ACaAuGT U cagAUCA
193	GCuUAuU U CaAUGGg	399	CAAUGUT C AgauCAA
134	CUUAUUU C AAUGgGA	399	CAAUGUT C AGAUCAA

399	CaAuGUU c agAl		658	CAGAUAU c	AcaaqAu
399	caAUGUU c aGAu	ICAA .	658 .	CAgauAU C	_
399	CAaUguU c aGAU	JcAa	658	CAGAUAU C	aCAAGAU
399	caauguu c agai	JCAA	658	CaGAUaU c	ACaAGau
399	CAaugUU c agAl	JCAA	666	aCAAGAU A	AUGUCAC
404	UUCAGAU C AAGO	EACA	666	ACAagaU a	AUGUCAC
404	UucAGaU c aAGC	ACa	671	AUaAuGU C	
418	aUGGGCU c GUAL	1gAU	671	aUAAUgU c	
418	AUGGGCU c GUAL	IgAu	671	AUAAUGU C	
418	AUggGCU c GUal	Ga U	682	gAACUgU u	
421	gGCUCgU a UGAL		683	aAcUGuU c	
421	ggCUCgU A UgAu	•	683	AAcUGuU c	
429	Ugauugu u uuau		691	aguaUcU C	
429	UGAUuGU u UUAL		691	agUAUCU c	
431	AuUgUuU u AUAc		691	aGUAucU C	_
431	AUUGUUU U AUaC		701	aCaGCcU c	
432	UuGUuUU A UaC		701	acagCCU c	
432	UuGUUUU a Uaca		703	AGCcUcU C	
432	uUGUUUU a uAca		703	aGCcUcU c	
461	gAUcaAU u AUCC	ncC	707	Ucucucu u	
462	AucaAUU a uCct		707	UcUCUcU u	
464	CAauUaU c CUcC		708	cucucuu u	
467	uUAUCcU C CAaC	-	709	CCCCCCC C	
467	UUauCcU C CAaC	_	709	UCUCUuU c	
467	UUaUccu c caac	-	709	UCUcUuU c	
467	UuAuCCU C CaaC		712	CUUUcaU U	
490	GAACUGU C AGUG		712	CUUUCAU U	_
497	CAGUGAU c GCCA		712	Cultucati u	-
505	GCCAACU U CAGU		712	CUUUCAU U	
506	CCAACUU C AGUg		712	CUUUCAU u	
506	CCAaCUU C aGUg		713	uuUCAUU c	
521	CUGAAAU A aaAC		713	UUUCAUU C	
531	ACUGgcU c AgAa		732	GuGgcAU a	
539	agaaUGU A ACAG	_	732	GuGgcAU A	
550	GgAaAuU c uGGC		740	UGACCGU u	
550	ggAAAUU C Uggc		749	UgUGUaU U	
557	CUGGCAU A AAUU		749	uGuGUGU U	
561	CAUAAAU U UGAC		750	guguguu c	
562	AUAAAUU U GACC			GUGUGUU C	
576	CaCgUCU A agCA			ugAAGaU U	
585	gCAaGGU c ACCC		778	aUUUccu c	
597	gaaaccu a agaa		788		
607	AaGaUgU a uUuU			AACCUCU C . UUUCACU c .	
611	UGUAUUU u cUgA	uAa		ChaghGU U	
625	ACUAAUU C AACU	Aau		CAAgAGU U	
630	UUCAACU A auGA			AAgAGJU u	
630	UUCAACU A Auga			_	
637	AauGAGU A UGgU			UUUCCAU C	
656	uGCAgaU a UcAc			uUUCcaU c :	
				uccaucti c :	LUCAAAC

836	aGgAGAU U acAGCUU
836	aggagau u acagcuu
837	GgAGAUU a cAGCUUc
848	CUUCAGU u AcugUGg
860	UGGCCeU C CUeCUug
860	UggCCcU c CUCcuUg
878	ugCUGCU C AUCAUUg
951	GCGGgaU a GuAACgC
974	AgaCuAU c aACCUGA
989	aGgaAcU U GaACCCc
1006	auUgCUU c aGCAAAa
1055.	AAAgAGU u aaAAaUU
1056	AaGAgUU a aaAAuUG
1062	uaaaaau u geuuugc
1092	CAgaGUU u CuCAGAA
1095	aGUUUcU c AgAaUUC
1101	UCAGAAU u caaAaAU
1101	ucAGAAU U CAAaaAU
1101	Ucagaau u caaaaau
1111	aAaAUGU U cUcAgcU
1112	AaAUGUU c UcAgeUg
1128	UUgGAaU u cuACAGU
1128	UUGGAaU u CuaCaGU
1131	GAAUUCU a cAGuUgA
1131	GAauUCU a CAguuGA
1141	GuUGAAU a aUuAAag
1144	gaaUAAU U AAAGAac
1145	AAuAaUU a aAgaACA

Table BIX: Mouse B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
47	GUIGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
74	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA ACACGUC
83	CUUCCGU CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
139	UGUCACA CUGAUGAGGCCGAAAGGCCGAA AGAUAAG
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
149	UCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACUGUCA
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
170	CCACGGA CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
171	UCCACGG CUGAUGAGGCCGAAAGGCCGAA AACAGCA
172	CUCCACG CUGAUGAGGCCGAAAGGCCGAA AAACAGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
190 192	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
194	UCCCAUU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG

194	UCCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAG
208		CUGAUGAGGCCGAAAGGCCGAA	
210		CUGAUGAGGCCGAAAGGCCGAA	
223		CUGAUGAGGCCGAAAGGCCCGAA	
223		CUGAUGAGGCCGAAAGGCCCGAA	
224		CUGAUGAGGCCGAAAGGCCGAA	
225		CUGAUGAGGCCGAAAGGCCGAA	
225		CUGAUGAGGCCGAAAGGCCGAA	
242		CUGAUGAGGCCGAAAGGCCCGAA	
260		CUGAUGAGGCCGAAAGGCCGAA	
260		CUGAUGAGGCCGAAAGGCCGAA	
263		CUGAUGAGGCCGAAAGGCCGAA	
263		CUGAUGAGGCCGAAAGGCCGAA	
265		CUGAUGAGGCCGAAAGGCCGAA	
265		CUGAUGAGGCCGAAAGGCCGAA	
266		CUGAUGAGGCCGAAAGGCCCGAA	
266		CUGAUGAGGCCGAAAGGCCGAA	
266		CUGAUGAGGCCGAAAGGCCGAA	
267		CUGAUGAGGCCGAAAGGCCCGAA	
267	UCCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
286	CAGAACC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUG
286	CAGAACC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUG
290		CUGAUGAGGCCGAAAGGCCGAA	
291	UCGUACA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAC
295	GUGCUCG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAC
304		CUGAUGAGGCCGAAAGGCCGAA	
307		CUGAUGAGGCCGAAAGGCCGAA	
323		CUGAUGAGGCCGAAAGGCCGAA	
343	GCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACTUUGGC
343		CUGAUGAGGCCGAAAGGCCGAA	
361		CUGAUGAGGCCGAAAGGCCGAA	
381		CUGAUGAGGCCGAAAGGCCGAA	
383		CUGAUGAGCCCGAAAGCCCGAA	
383	GAAGUCG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUCC
389	CAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUA
389		CUGAUGAGGCCGAAAGGCCGAA	
390		CUGAUGAGGCCGAAAGGCCGAA	
390		CUGAUGAGGCCGAAAGGCCGAA	
398		CUGAUGAGGCCGAAAGGCCGAA	
398		CUGAUGAGGCCGAAAGGCCGAA	
398		CUGAUGAGGCCGAAAGGCCGAA	
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACADUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACADUG
399		CUGAUGAGGCCGAAAGGCCCGAA	
399	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AACADUG
404	UGUCCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA

404	UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
418	AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418	AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418	AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
421	ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
421	ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
429	UGUAUAA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
429	UGUAUAA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
431	UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
431	UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
432	UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432	UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432	UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
461	GGAGGAU CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
462	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
464	GUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAAUUG
467	UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
490	GAUCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
497	AGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCACUG
505	UUCACUG CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
506	GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
- 506	GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
521	CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
531	ACAUUCU CUGAUGAGGCCGAAAGGCCGAA AGCCACU
539	UUCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
550	UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC
550	UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC
557	UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
561	CAGGUCA CUGAUGAGGCCGAAAGGCCCGAA AUTITIATIC
562	GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUULAU
576	CCUUGCU CUGAUGAGGCCGAAAGGCCGAA AGACGUG
585	UUCGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGC
597	AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
607	CAGAAAA CUGAUGAGGCCGAAAGGCCGAA ACADCUU
611	UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAAUACA
625	AUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUAGU
630	UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
630	UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
637	AUCACCA CUGAUGAGGCCGAAAGGCCGAA ACUCAIIII
656	CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGCA
658	AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUKTIS
658 650	AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 650	AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658	AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUKTIS
666	GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGTI
666	GUGACAU CUGAUGAGGCCGAAAGGCCCGAA AUCUUGU

671	GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAU
671	GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAL
671	GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAL
682	GAUACUG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
683	AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
683	AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
691	GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691	GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691	GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
701	AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGAUACU
701	AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
703	UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
703	UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
707	GGANGA CICANAGCCUAA AGAGGCU
707	GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
708	GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
709	GGGAAUG CUGAUGAGGCCGAAAAGGCCGAA AAGAGAG
709	CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
709	CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
712	CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
712	AUCCOGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712	AUCCOGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712	AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712	AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
713	AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
713	CAUCCGG CUGAUGAGGCCGAAAAGGCCGAA AAUGAAA
732	CAUCOGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
732	ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
740	ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
749	CACACAC CUGAUGAGGCCGAAAGGCCGAA ACCGUCA
749	UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
750	UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
750	GUUUCCA CUGAUGAGGCCGAAAGCCCGAA AACACAC
773	GUUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC
778	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
788	AGGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAAAU
798	UGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
805	AACUCUU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
805	AGAUGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
806	AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
811	GAGAUGG CUGAUGAGGCCGAAAAGGCCGAA AACUCUU
811	UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
813	UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
836	GUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGA
836	AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
837	AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
848	GAAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCUCC
860	CCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG
860	CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA
	CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA

878	CAAUGAU CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
951	GCGUUAC CUGAUGAGGCCGAAAGGCCGAA AUCCCGC
974	UCAGGUU CUGAUGAGGCCGAAAGGCCGAA AUAGUCU
989	GGGGUUC CUGAUGAGGCCGAAAGGCCGAA AGUUCCU
1006	UUUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGCAAU
1055	AAUUUUU CUGAUGAGGCCGAAAGGCCGAA ACUCUUU
1056	CAAUUUU CUGAUGAGGCCGAAAGGCCGAA AACUCUU
1062	GCAAAGC CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
1092	UUCUGAG CUGAUGAGGCOGAAAGGCOGAA AACUCUG
1095	GAAUUCU CUGAUGAGGCCGAAAGGCCGAA AGAAACU
1101	AUUUUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
1101	AUUUUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
1101	ATTITUTE CICATER COCCER & COCCER & AUUCUGA
1111	AUJUUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
1112	AGCUGAG CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
1128	CAGCUGA CUGAUGAGGCCGAAAGGCCGAA AACAUUU
1128	ACUGUAG CUGAUGAGGCCGAAAGGCCGAA AUUCCAA
1131	ACUGUAG CUGAUGAGGCCGAAAGGCCGAA AUUCCAA
1131	UCAACUG CUGAUGAGGCCGAAAGGCCGAA AGAAUUC
1141	UCAACUG CUGAUGAGGCCGAAAGGCCGAA AGAAUUC
1141	CUUUAAU CUGAUGAGGCCGAAAGGCCGAA AUUCAAC
1144	GUUCUUU CUGAUGAGGCCGAAAGGCCGAA AUUAUUC
TT42	UGUUCUU CUGAUGAGGCCGAAAGGCCGAA AAUUAUU

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Table BX: Human CD40 Hammerhead Ribozyme Target Sequences

9 CCUCGCU C GGGGGCC 449 UUGGGGU C AAGCAGA 244 CAGUGGU C CUGCGCC 449 AGCAGAU U GCUACAG 377 GCCUGGU C UCACCUC 453 GAUUGCU A CAGGGGU U UCUGAUA 444 CUCACCU C GCCAUGG 461 CAGGGGU U UCUGAUA 444 CUCACCU C GCCAUGG 462 AGGGGUU U CUGAUAC 53 CCAUGGU C GCCAUGG 463 GGGGUU U CUGAUAC 53 CCAUGGU C GUCUCCC 463 GGGGUU C UGAUACC 54 CAUGGGU C GUCUGCC 468 UUCUGAU A CCAUCUG 57 GGUUCGU C UGCCCCU 473 AUACCAU C UCCAGGC 63 UCUGCGU C UGCCCCU 473 AUACCAU C UCCAGGC 63 UCUGCGU C UGCCCCU 473 AUACCAU C UCCAGGC 63 UCUGCGU C UGCGCGU 496 GUCGGCU U CUUCUCC 663 UCUGCCU C UGGGGCU 497 UCCGCCU U CUUCUCC 777 GCGUCCU C UGGGGCU 499 GGCUUCU U CUCCCAU 688 GGCUGCU U GCUGAAC 499 GGCUUCU U CUCCCAU 688 GGCUGCU U GCUGACC 499 GGCUUCU U CUCCCAU 688 GGCUGCU U GCUGAACC 500 GCUUCUU C UCCAAUG 105 UGUCCAU C CAGAACC 500 GCUUCUU C UCCAAUG 105 UGUCCAU C CAGAACC 500 GCUUCUU C UCCAAUG 1139 AAACAGU A CCUAAUA 511 AAUGUC U UCUGCC CAAUGUG 1144 AGUGCGU U CUUCUCC CAAUGUG 1153 AAACAGU A ACAGGUC 519 AUCUGCU U UCGAAAAA 1146 ACCUAAU A AACAGUC 519 AUCUGCU U UCGAAAAA 1162 GUGCCUU U UUGUCGC 521 CUCUUCU C GAAAAAA 1163 UGCUGUU U UUGUGCC 521 CUCUCUU U CGAAAAA 162 GUGCCUU U UGGAAAAA 162 GUGCCUU U UGGAAAAA 162 GUGCCUU U UGUGCAA 566 ACCUGGU U UGUGCAA 566 ACCUGGU U GGGCAAC 566 ACCUGGU U GUGCAAC 567 AUCACUU U GCGAACU C CUCCAGAA 667 UCCCCCU U GCGAAC 667 UCCCCC	nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
24 CAGUGGU C CUGCCGC 449 AGCAGAU U GCUACAG 37 GCCUGGU C VCACCUC 453 GAUGGU A CAGGGGU 38 CUGGUCU C ACCUGC 461 CAGGGGU U UCUGAUA 44 CUCACCU C GCCAUGG 462 AGGGGUU U CUGAUAC 53 CCAUGGU U GGUCUGC 463 GGGGUUU C UGAUACC 54 CAUGGUU C GUCUGC 468 UUCUGAU A CCAUCUG 55 CGAUGGU C UGCCUCU 473 AUACCAU C UGCGAGG 63 UCUGCCU C UGCAGUG 491 GCCCAGU C GGCUUCU 74 AGUGCGU C UGCAGUG 496 GUCGCCU U UCUCCAC 88 GGCUGCU U GCUGACC 499 GGCUUCU U UCUCCA 88 GGCUGCU U GCUGACC 499 GGCUUCU U UCUCCA 101 CCGCCUG C CAUCCAG 500 GCUUCUU C UCCCAUC 102 GGCUGCU C UGCGGCU 497 UCCGCUU C UCCCAUC 103 AAACAGU C CAGAACC 502 UUCUUCU C CAAUGUG 139 AAACAGU A CCUTAUA 511 AAUGUGU C UCCCAU 143 AGUACCU A AUAAACA 514 GUGUCAU C UCCGAUAC 146 ACCUTAU A AACAGUC 519 AUCUGCU U UCUGCCU 146 ACCUTAUA A AACAGUC 519 AUCUGCU U UCGAANA 146 ACCUTAU A AACAGUC 519 AUCUGCU U UCGAANA 146 ACCUTAU A AACAGUC 520 UCUCUCU U GAAAAAA 146 ACCUTAU A AACAGUC 520 UCUCCUU U GAAAAAA 162 GUGCUGU U UUUGUGC 521 CUGCCUU U GAAAAAA 163 UGCUGUU U UGUGCCA 537 UCACCCU U GAAAAAA 164 ACCUTAU C AGUGCCAG 566 ACCUGGU U GUUCGUU 165 CUGUUCU U UGUGCCAG 566 ACCUGGU U GUCCAAUC 166 UGUUCUU U UGUGCCAG 566 ACCUGGU U GUCCAGU 208 ACAGAGU U CACUGAAA 599 CUGAUGU U GGCCAAC 208 ACAGAGU U CACUGAAA 599 CUGAUGU U GUCGCAAC 208 ACAGAGU U CACUGAA 599 CUGAUGU U GUCCAAC 209 CAGAGUU C ACUGAAA 602 AUGUUGU C UGUGGUC 227 AAUGCCU U CUUCGCG 609 CUGCGU C GCCAGGA 228 AUGCCUU C CUUGCGG 618 CCCGGAU C GCCUGGG 228 AUGCCUU C CUUGCGG 618 CCCGGAU C GCCUGGA 229 CAGAGUU C CUUGCGG 659 CUGGGUU C CUCCGUG 221 AAUCCCU U CCGGGCC 659 UCGGGAU C CCCAUCA 248 GCGAAUU C CUGAGAC 650 CCAUCAU C UUCGGGA 251 AAUCCCU A GGCCUGA 665 UCCUGUU C GGGAUCC 252 AAUCCCU C CUGGGGC 331 CCUUCCU A GGCCUGA 665 UCCUGGU C CCCAUCA 3320 UUCGGGU C CAGAACA 671 UUGCCAU C UUCGGGA 3331 GCCACCU C AGAACAA 674 CCAUCCU U UGCGAUC 3333 ACACCCU C AGAACAA 674 CCAUCCU U UGCGGAU 348 CCACCU C AGAACAA 674 CCAUCCU U UGCCAUC 348 CGGAAUU C CUGAGAC 665 UCCUGGUU C UUCGCGGA 340 CCACCCU C AGAACAA 674 CCAUCCU U UAUCAAA 3418 CCACCCU C AGAACAA 674 CCAUCCU U UAUCAAA 4418 CACCGCU C AGAACAC 669 UCCUUUAU C AAAAAAGG	9	CCUCGCU C GGGGGGC	440	UNGGGGI C AAGCAGA
37 GCCUGGU C UCACCUC 453 GAUUGCU A CAGGGGU U UCUGAUA 44 CUCACCU C GCCAUGG 461 CAGGGGU U UCUGAUAC 53 CCAUGGU C GCCAUGG 462 AGGGGUU C UGAUACC 53 CCAUGGU C UGCUCC 463 GGGGGUU C UGAUACC 54 CAUGGUU C UGAUACC 468 UUCUGAU A CCAUCUG 57 GGUUCGU C UGCUCCU 473 AUACCAU C UGCCAGG	24			
39 CUGGICU C ACCUGC 461 AGGGGIU U UCUGAUA 444 CUCACCU C GCCAUGG 462 AGGGGIU U CUGAUAC 53 CCAUGGU U GUCUGC 54 CAUGGUU C GUCUGC 55 GGUUCGU C GUCUGC 56 UCUGCU 468 UUCUGAU A CCAUCUG 57 GGUUCGU C UGCAGUG 63 UCUGCCU C UGCAGUG 64 GGUCGCU C UGCAGUG 65 UCUGCU C UGCAGUG 67 UCUGCGU C UGCAGUG 68 GCCAGU C GCCUCU 68 GGCUCU U CUUCUCC 68 GGCUCU U GCCGAGU 68 GGCUCU U CUCUCCA 68 GGCUCU U CUCCCAAU 68 GGCUCU U CUCCCAAU 68 GGCUCU U CUCCCAAU 69 GGCUUCU U CUCCCAAU 69 GGCUUCU U CUCCCAAU 60 GCUUCU U CUCCCAAU 61 CCGCAGU C CAUCCAG 61 UUCUUCU C CAAUGA 61 CCGCAGU C CAUCCAG 62 UUCUUCU C CAAUGA 63 UCUGCU C CAUCCAG 64 GCCUCU U CUCCCAAU 65 GCUUCU U CUCCCAAU 66 GCUUCU U CUCCCAAU 67 GCGUCU U CUCCCAAU 68 GGCUGCU U CUCCCAG 68 GCUUCU U CUCCCAAU 68 GCCUGCU A AUAAACA 614 GUGUCAU C CAGAACC 650 UCUCCCU U CCGAAUG 651 AAACAGU C AGUGCUG 652 UCUCUCU U CGAAAAA 651 GUGUCGU U UCUCUCU C CAAAAA 651 GUGCUGU U UUUGUG 651 AAACAGU C AGUGCUG 652 UCUCUCU U GAAAAA 651 AAACAGU C UUUGUG 651 AAACAGU C UUUGUG 652 UCUCUCU U GGAAAAA 653 UCGCUGU U UUUGUG 6531 AAAAAGU C ACCCAUG 666 ACCUGGU U GUGCAAC 667 ACCUGGU U GUGCAAC 668 ACAGAGU U GACCAAA 660 ACAGAGU U GUGUGAA 660 ACAGAGU U GCCUGAA 660 ACAGAGU U GCCUGAA 660 ACAGAGU U CACUGAA 660 ACAGAGU U CUUCGG 660 CUGUGGU C CCCAGGA 661 CCUCCU U GCGGGAA 662 AUGUUGU C UUGCGG 663 ACAGAGU U CCUGAGA 664 AUGCCCU U CCUGCGG 670 CUGUGGU C CCCAGGA 671 UCGCGAU C UUCGGG 671 AGGGAU U CCUGAGA 672 AGGGAU U CCUGAGA 673 ACAGAGU U CCUGAGA 674 AGGGAU U CCUGAGA 675 AUGCCCU U CCGGGAA 676 AUCCCUU U GCGGAU 677 AGGGAU U CCUGAGA 678 ACAGAGU U CCUGAGA 679 CUGUGU C UUCGGG 688 CCAACCU A GGCUGC 664 AUCCCUGU U UCGCGAA 670 CCAUCAU U UCGCGAA 671 UCCCCAU C UUCGGG 671 AUGCCCU U CCGGAU 672 AUGCCU U UCGCGAA 673 AUGCCCU U CCGGAU 674 AGGGAU U CCUGAGA 675 AUCCCUU U UCGCGAA 676 AUCCCUU U UCGCGAA 677 AGGGAU U CCUGAGA 677 AGGGAU U CCUGAGA 678 AUGCCUU U UCGCGAA 679 CUGUGU U UCCCAAU 679 AGGAAU U CCUGAGA 670 CCUUCU U UCGCGAA 671 UCCCAU U UCCCAAU 671 AUCCCAU U UCCCAAU 672 AUCCUU U UCCCAAU 673 AUCCCUU U UCCCAAU 674 AGGCAU U UCCCAAU 675 AUCCUU U UCCCAAU 676 AUCCUU U UCCCAAU 677 AGGAA	37	GCCUGGU C UCACCUC	453	
44 CUCACCU C GCCALIGG 462 AGGGGUIU U CUGAUAC 53 CCALIGGU U CUGUCUGC 468 UUCUGAU A CCAUCUG U CUGUGCC 468 UUCUGAU A CCAUCUG U CAUGGUU C UGCGGGC 468 UUCUGAU A CCAUCUG U CUGCGGC 63 UCUGCCU C UGCCAGUG 491 GCCCAGU C GGCUUCU C UGCGGGC 496 GUCGGCU U CUUCUCC AGGGCUCU C UGCGGGC 497 UCGGCUU C UCUCCCA 499 GGCUUCU U CUCCCAU C GGCUCU U CUCCCAU C UGCGGCU C CAUCAGA 500 GCUUCUU C UCCCAAU C UGCGCUC C CAUCAGA 500 GCUUCUU C UCCCAAU C UGCGCUU C CAAUGUG 139 AAACAGU C CAAAACC 502 UUCUCUC C CAAUGUG 139 AAACAGU A AUTAACAA 511 AAUGUGU C UCCAAUG 146 ACCUAAU A AUTAACA 511 AAUGUGU C UCCAAUA 146 ACCUAAU A AUTAACA 511 GUGUCCU U UCGGAAAA 153 AAACAGU C AGGGCUUC U UCGGAAAA 153 AAACAGU C AGUGCCC 520 UUCUGCU U UCGAAAAA 162 GUGCUGU U CUUUGUG 521 CUCUCUU C GAAAAAA 162 GUGCUGU U UUUGUGC 531 AAAAUGU C ACCCUUG U UUGUGC 531 AAAAUGU C ACCCUUG U UUGUGCC 531 AAAAUGU C ACCCUUG U UUGUGCC 531 AAAAUGU C ACCCUUG 166 UGUUCUU U UGUGCCA 537 UCACCCU U GGAAAAA 602 AUGUCCU U UGUGCCA 534 AUGUCCU U GGAAAAA 602 AUGUCCU U GGAAAAA 602 AUGUCCU U GGAAAAA 602 AUGUCCU U GGAAAAA 602 AUGUCCU U CUUCCG 609 CUGUGGU C CCCAGGA 228 AUGCCUU C CUUGCG 618 CCAGGAU C CCCAUGA 229 CAGAAUU C CUUGCG 618 CCAGGAU C CCCAUGA 229 CAGAAUU C CUUGCG 618 CCAGGAU C CCCAUGA 229 CACAAAU A CUGCGG 618 CCAGGAU C CCCAUCAU C UUCGGG 618 CCAGGAU C CUUCUCU U CGGGAUCC 664 AUCCCCU U CGGGUCC 664 AUCCCCU U CGGGUCC 664 AUCCCCU U CGGGUCC 664 AUCCCCU U CGGGUCC 665 UCCCCGU C UUCGGGU C CCCACCC 688 CUCCUU U AUCAAAA 641 CCACCCU U UCGGGUC C CCCACCC 688 CUCCUU U AUCAAAA 641 UCAGGCU C C	39	CUGGUCU C ACCUGGC	461	
53 CCANGGU U CGUCUGCC 468 UUCUGAU A CAUCUG 54 CAUGGU C GUCUGCC 468 UUCUGAU A CAUCUG 55 GGUUCGU C UGCAGUG 491 GCCCAGU C GGGUUCU U 74 AGUCCGU C UGCAGUG 491 GCCCAGU C GGGUUCU 75 GGUCCCU C UGCGGCU 497 UCCGCAU C UUCUCCA 68 GGCUGCU U GGUGCCC 499 GGCUUCU U CUCCAAU 68 GGCUGCU U GCUGACC 499 GGCUUCU U CUCCAAU 68 GGCUGCU C CAUCCAG 500 GCUUCUU C UCCAAUG 69 GUCCCAU C CAGAACC 500 UUCUUCU C CAAUGUG 60 GCUCCU C CAGAACC 500 UUCUUCU C CAAUGUG 61 AAACAGU A AUAAACA 511 AAUGUGU C UCCAAUG 61 ACCUAAU A AACAGUC 519 AUCUGCU U UCCAAUG 62 GUGCUGU U CUUUGUG 520 UUCUGCU U CAAAAAA 63 AAACAGU C AGUGCUG 520 UUCUGCU U CAAAAAA 64 ACCUAAU A AACAGUC 519 AUCUGCU U UCAAAAA 65 ACCUAAU A CAGAGUC 520 UUCUGCU U CAAAAAA 66 ACCUAAU A AACAGUC 519 AUCUGCU U UCAAAAA 66 ACCUAGU U CUUUGUG 521 CUGCUUU C GAAAAAA 67 ACCUGUU U UUUGUG 521 CUGCUUU C GAAAAAA 68 ACAGAGU U CACUGAA 599 CUGCUGU U GGACAAG 68 ACAGAGU U CACUGAA 599 CUGAUGU U GGACAAG 68 ACAGAGU U CACUGAA 599 CUGAUGU U GUCUGUG 69 CAGAGUU C ACUGAAA 602 AUGUUGU C UGUGGUC 609 CAGAGUU C ACUGAAA 602 AUGUUGU C GCCCAGA 68 ACAGAGU U CACUGAA 609 CUGUGGU C CCCAGGA 68 ACAGAGU U CCUUGCG 609 CUGUGGU C CCCAGGA 68 ACGAAAU C CUUGCGG 618 CCAGGAU C GCCUGG 69 CUGUGCU C CUUGCGG 618 ACAGAGU U CCUGCGG 619 CUGUGGU C CUGUGGG 610 CCAUCAU C GGGUCA 647 UCCCCAU C CUUCCGG 618 CCAGGAU C CCCAUCA 648 GCGAAUU C CUUGCGG 619 CCGCCAU C CUGUGGG 610 CCAUCAU C GGGUCC 640 AUCCUCU U GCGGUCC 641 UGGGGAU C CCCAUCA 642 UCCCCAU C AGAACC 653 UCCAUCAU U CCGGUCC 664 AUCCUCU U GCGGAU C CUGUUGG 618 CCAGCAU C CUGUGGG 618 CCAGCAU C CUGCGG 618 CCAGCAU C CUGUGGG 619 CUGUGUU U UGCCGU C UGCGGGA 647 UCCCCAU C CUGUGGG 618 CCAGCAU C CUGUGGG 618 CCAGCAU C CUGUGGG 618 CCAGCAU C CUGUGGG 619 UCGCCUU U GCGGUCU U UAUCAA 640 CCAGCCU C AGAACC 665 UCCUCU U UGCGGUU U UAUCAA 650 UCUUCU U UGCGGCU C CAGCCC 688 CUGCUU U AUCAAAA 666 UCCUCUU U UGCGGUC 669 UCCUUU U A		CUCACCU C GCCAUGG	462	
CAUGGUI C GUCUGCC 468		CCAUGGU U CGUCUGC	463	
63 UCUGCCU C UGCABUG 491 GCCCAGU C UGCGAGC 63 UCUGCCU C UGCABUG 491 GCCCAGU C GGCUUCU 77 AGUGCGU C UGCAGUG 496 GUCGGCU U CUUCUCC 77 GCGUCCU C UGGGGCU 497 UCGGCUU C UUCUCCC 88 GGCUGCU U GCUGACC 499 GGCUUCUU C CUCCAAU 101 CCGCUGU C CAGCACC 500 GCUUCUU C CCCAAU 105 UGUCCAU C CAGCACC 500 GCUUCUU C CCAAUGU 139 AAACAGU A CCUAAUTA 511 AAUGUGU C UGCAAU 146 ACCUAAU A AACAGUC 519 AUCUGCU U UCGAAAA 153 AAACAGU C AGUGCUG 520 UCUGCUU U CGAAAAA 153 AAACAGU C AGUGCUG 520 UCUGCUU U CGAAAAA 153 AAACAGU C AGUGCUG 520 UCUGCUU U CGAAAAA 164 ACCUAAU A AACAGUC 519 AUCUGCU U UCGAAAA 165 GUGCUGU U CUUUGUG 521 CUGCUUU C GAAAAAA 166 UGUUCUU U UUGUGC 531 AAAAUGU C ACCUUG 166 UGUUCUU U UUGUGCC 531 AAAAUGU C ACCUUG 166 UGUUCUU U UUGUGCC 537 UCACCCU U GGACAAA 166 UGUUCUU U UUGUGCC 537 UCACCCU U GGACAAC 166 UGUUCUU U UUGUGCCA 537 UCACCCU U GGACAAC 208 ACAGAGU U CACUGAA 599 CUGAUGU U GUGCAAC 209 CAGAGUU C ACUGAAA 599 CUGAUGU U GUCUGUG 209 CAGAGUU C ACUGAAA 599 CUGAUGU U GUCUGUG 209 CAGAGUU C ACUGAAA 599 CUGAUGU U GUCUGUG 227 AAUGCCU U CCUUGCGG 609 CUGUGGU C CCCAGGA 231 CCUUCCU U GCGGUGA 641 UGGGAAU C CCCAUCA 248 GCGAAUU C CUUGCGG 618 CCAGGAU C GGCUGAG 251 AUGCCUU C CUUGCGG 618 CCAGGAU C CCCAUCA 248 GCGAAUU C CUUGCGG 668 CCAGGAU C CCCAUCA 248 GCGAAUU C CUUGCGG 669 CUGUGGU C CCCAGGA 251 AAUGCCU U GCGGUGA 641 UGGGAAU C CCCAUCA 248 GCGAAUU C CUAGACA 650 CCAUCAU C UUCGGGA 251 AAUGCCU A GACACCU 652 AUCAUCU U CGGGAUC 252 AAUGCCU C GGGUCCA 665 UCCUGUU U GGGAUC 263 UCUCGGU C GGGUCCA 666 UCCUGU U UGCCAUC 314 UAGGGCU C CGGGCCC 664 AUCCUGU U UGCCAUC 315 AGGGCUU C GGGUCCA 665 UCCUGUU U UGCCAUC 337 GGCACCU C AGAAACA 671 UCCCCAU C UUCGGGA 338 CCAACCU A GGCCUCC 664 AUCCUCU U GGGAUCC 339 UUCGGGU C CAGCAGA 671 UUCCCAU C UUUGGG 330 CCAACCU A GGCCUCC 664 AUCCUCU U GGGAUCC 331 UCAGCGU C CAGCAGA 671 UUCCCAU C UUUGCG 331 CCUUCUU A CGGGUCC 668 UCCUCGU U UACCAAAA 407 GCUGUU A CGACCC 688 UCGUCU U AUCAAAA 407 GCUGUGU C CUGCACC 688 UCGUCU U AUCAAAA 408 CCCGCCU C AUGCUCC 669 UCGUUU A AUCAAAA 409 UCUUCAU C GCCCGCC 669 GGCCUUU A ACAAAAA 400 UCAUCCU C GCCCGCC 669 UCCUUUAU C			468	
100 100	= -		473	
74 AGUGCGU C CUCUGGG 496 GUCGGCU U CUUCUCC 777 GCGUCCU C UGGGCCU 497 UCGGCUU C UUCUCCA 88 GGCUGCU U GCUGACC 499 GGCUUCU U CUCCAAU 101 CCGCUGU C CAUCCAG 500 GCUUCUU C UCCAAU 105 UGUCCAU C CAGAACC 502 UUCUUCU C CAAUGUG 139 AAACAGU A CCUAAUA 511 AAUGUGU C AUCUCCU 146 ACCUAAU A AUCAACA 514 GUGUCAU C UGCAAUA 146 ACCUAAU A AACAGUC 519 AUCUCCU U CGAAAAA 153 AAACAGU C AGUGCCG 520 UCUGCUU U CGAAAAA 153 AAACAGU C AGUGCCG 520 UCUGCUU U CGAAAAA 162 GUGCUGU U CUUUGUG 521 CUGCUUU C GAAAAAA 163 UGCUGUU C UUUGUGC 521 CUGCUUU C GAAAAAA 164 UGCUGUU U UUUGUGC 531 AAAAAGU C ACCCUUG 165 CUGUUCUU U UGUGCCA 537 UCACCCU U GGACAAA 166 UGUUCUU U UGUGCCA 537 UCACCCU U GGACAAC 208 ACAGAGU C ACUGAAA 599 CUGAUGU U GUGCCAG 209 CAGAGUU C ACUGAAA 602 AUGUUGU C UUUGCUG 227 AAUGCCU U CCUUGCG 609 CUGUGGU C CCCAGGA 228 AUGCCUU C CUUGCGG 618 CCAGGAU C GGCUGAG 228 AUGCCUU C CUUGCGG 618 CCAGGAU C GCCUAGA 247 AGCGAAU C CCUAGAC 647 UCCCCAU C AUGUUCG 248 GCGAAUU C CUUAGAC 650 CCAUCAU C UUCGGGA 251 AAUUCCU A GACACCU 652 AUGUUCU C GGGAUCC 264 GCGAAUU C CUAGACC 651 UCAUCCU C CUCAGGA 265 ACGGAUU C CUAGACC 651 UCAUCCU U CGGGAUC 274 AGCGAAU A CUGCGAC 651 UCAUCCU C GGGAUCC 275 AAUUCCU A GACACCU 652 AUCAUCU C GGGAUCC 276 AAUGCCU C CUAGACC 651 UCAUCCU C GGGAUCC 277 AGCGAAU A CUGCGAC 651 UCAUCCU C GGGAUCC 278 AUGCCUU C GGGUCC 664 AUCCUCU U CGGGAUC 279 CACAAAU A CUGCGAC 651 UCAUCCU C GGGAUCC 270 AAUUCCU A GACACCU 652 AUCAUCU U CGGGAUC 270 CACAAAU A CUGCGAC 651 UCAUCCU C UUCGGGA 271 AAUUCCU A GACACCU 652 AUCAUCU U CGGGAUC 272 CACAAAU A CUGCGAC 665 UCCUGUU U GCCCAUCAU 315 AGGGCUU C GGGUCCA 665 UCCUGUU U GCGGAUC 316 CCACCUU A GGACCCU 668 UCCUGGU U UCCCAUC 317 CGCCCUU C GGGUCCA 665 UCCUGUU U UCCCAUCA 318 CCACCUU A GGGCUCC 668 UCCUGGU U UCCCAUCA 319 CCCCUGU C CUGCACC 688 CUGCUCU U UCCCAAA 310 CCCCGCU C AGAAACA 671 UCCCCAU C UUCCGAA 311 CCCCCUGU C CUGCACC 688 CUGCUCU U AUCAAAA 407 CCCCGGCU C AGAAACA 674 CCAUCCU U UCCCAAAAAA 407 CCCCGGCU C AGGGUCC 688 CUGCUU U AUCAAAA 408 CCCGGCU C AUGCUCC 689 UCGUCUU U AUCAAAA 400 CCCCGGCU C AGGGUCC 669 UCCUUCAU C AAAAAGG			491	
88 GGCUGCU U GCUGACC 499 GGCUUCU U CUCCAAU 101 CCGCUGU C CAUCCAG 500 GCUUCUU C UCCAAU 105 UGUCCAU C CAGAACC 502 UUCUUCU C CAAUGUG 139 AAACAGU A CCUAAUA 511 AAUGUGU C AUCUGCU 143 AGUACCU A AUAAACA 514 GUGUCAU C UGCUUUC 144 ACGUCAU A AUAAACA 514 GUGUCAU C UGCUUUC 153 AAACAGU C AGUGCUG 520 UCUGCUU U UGAAAAA 153 AAACAGU C AGUGCUG 520 UCUGCUU U CGAAAAA 162 GUGCUGU U UUUGUG 521 CUGCUUU C GAAAAA 163 UGCUGUU C UUUGUGC 531 AAAAUGU C ACCCUUG 165 CUGUUCU U UGUGCCA 537 UCACCCU U GGACAAG 166 UGUUCUU U UGUGCCA 537 UCACCCU U GGACAAG 166 UGUUCUU U UGUGCCA 537 UCACCCU U GGACAAG 208 ACAGAGU U CACUGAA 599 CUGAUGU U GUCUGUG 209 CAGAGUU C ACUGAAA 602 AUGUUGU C UGUGUG 227 AAUGCCU U CCUUGCG 609 CUGUGU C UGUGUG 228 AUGCCUU C CUUGCG 618 CCAGAAU C GCCUGAG 231 CCUUCCU U GCGGUGA 641 UGGUGGU C CCCAUCA 247 AGCGAAU C CUUGCG 647 UCCCCAU C AUCUCGG 248 GCGAAUU C CUUGCG 647 UCCCCAU C AUCUCGG 248 GCGAAUU C CUUGCG 652 AUCAUCU C GCGAGA 247 AGCGAAU C CUAGACA 650 CCAUCAU C UUCGGG 248 GCGAAUU C CUAGACA 650 CCAUCAU C UUCGGGA 251 AAUUCCU A GACACCU 652 AUCAUCU U CGGGAUC 252 AUCACCU A GACACCU 653 UCAUCUU C GGGAUCC 252 ACAAAAU A CUGCGAC 653 UCAUCUU C GGGAUCC 253 ACACCU A GGCUUCC 664 AUCCUGU U CGGGAUC 251 AAUUCCU A GACACCU 659 UCGGGAU C CUCUUGG 308 CCAACCU A GGCUUC 659 UCGGGAU C CUCUUGG 314 UAGGGCU C CAGCAGA 671 UUCCCCAU C AUCUUCG 315 AGGCCUU C GGGUCCA 665 UCCUGUU U GCGGAUC 316 AGGCCUU C GGGUCCA 665 UCCUGUU C GGGAUCC 317 AGCGCAU C CAGCAGA 671 UUCCCCAU C UUCGGGA 318 GCACCU C AGAAACA 674 CCAUCCU U GGGAUCC 319 ACACCAU A CUGCGAC 668 UCCUCGU U UUCAAAA 407 GCCCGCU C AGAACCU 668 CUGCUCU U UUCAAAA 407 GCCCGUC C AGCACCU 676 AUCCUCU U GGCCCUCU U UUCAAAA 407 GCCCGUC C AUCCUC 688 CUGCUCU U UUCAAAA 407 GCCCGCU C AUCCUCG 689 UCGUCUU U AUCAAAA 408 CCCCGCU C AUCCUCC 669 UCCUUU U AUCAAAA 409 GCCCCCCCCCC 669 UCCUUUAU C AAAAAAA			496	
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292 CACAAAU A CUGCGAC 653 UCAUCUU C GGGAUCC 308 CCAACCU A GGGCUUC 659 UCGGGAU C CUGUUUG 314 UAGGGCU U CGGGUCC 664 AUCCUGU U UGCCAUC 315 AGGGCUU C GGGUCCA 665 UCCUGUU U GCCAUCC 320 UUCGGGU C CAGCAGA 671 UUGCCAU C CUCUUGG 337 GGCACCU C AGAAACA 674 CCAUCCU C UUCGUGC 3381 GCACCAU C UGCACCU 676 AUCCUCU U GGUGCUG 381 GCACUGU A CGAGUGA 686 UGCUGGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 418 CACCGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCCGGCU U UGGGGUCC 692 UCUUUAU C AAAAAGG				
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315 AGGGCUU C GGGUCCA 665 UCCUGUU U GCCAUCC 320 UUCGGGU C CAGCAGA 671 UUGCCAU C CUCUUGG 337 GGCACCU C AGAAACA 674 CCAUCCU C UUGGUGC 353 ACACCAU C UGCACCU 676 AUCCUCU U GGUGCUG 381 GCACUGU A CGAGUGA 686 UGCUGGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG				
320 UUCGGGU C CAGCAGA 671 UUGCCAU C CUCUUGG 337 GGCACCU C AGAAACA 674 CCAUCCU C UUGGUGC 353 ACACCAU C UGCACCU 676 AUCCUCU U GGUGCUG 381 GCACUGU A CGAGUGA 686 UGCUGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCCGCCU U UGGGGUC 692 UCUUUAU C AAAAAGG	315			
GCACCU C AGAAACA 674 CCAUCCU C UUGGUGC 353 ACACCAU C UGCACCU 676 AUCCUCU U GGUGCUG 381 GCACUGU A CGAGUGA 686 UGCUGGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCCGCCU U UGGGGUC 692 UCUUUAU C AAAAAGG	320			
ACACCAU C UGCACCU 676 AUCCUCU U GGUGCUG 381 GCACUGU A CGAGUGA 686 UGCUGGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCCGCCU U UGGGGUC 692 UCUUUAU C AAAAAGG	337		- · -	
381 GCACUGU A CGAGUGA 686 UGCUGGU C UUUAUCA 407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG	353		- · -	
407 GCUGUGU C CUGCACC 688 CUGGUCU U UAUCAAA 418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG	381	GCACUGU A CGAGUGA		
418 CACCGCU C AUGCUCG 689 UGGUCUU U AUCAAAA 424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG	407			
424 UCAUGCU C GCCCGGC 690 GGUCUUU A UCAAAAA 433 CCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG	418			
433 CCCGGCU U UGGGGUC 692 UCUUUAU C AAAAAGG	424			
AZA OCOUCAU C AAAAAGG	433			
ANCEAN A MINITER	434	CCGGCUU U GGGGUCA	720	AACCAAU A AGGCCCC

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GGAGACU U UACAUGG

GAGACUU U ACAUGGA

AGACUUU A CAUGGAU

AACCGGU C ACCCAGG

AGAGAGU C GCAUCUC

GUCGCAU C UCAGUGC

CGCAUCU C AGUGCAG

AGGCAGU U GGCCAGA

GGGAGCU A UGCCCAG

GCCCAGU C AGUGCCA

Table BXI: Human CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
9	GGCGCCC CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
24	GCGGCAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
37	GAGGUGA CUGAUGAGGCCGAAAGGCCGAA ACCAGGC
39	GCGAGGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
44	CCAUGGC CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
53	GCAGACG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
54	GGCAGAC CUGAUGAGGCCGAAAGGCCGAA AACCAUG
57	AGAGGCA CUGAUGAGGCCGAAAGGCCGAA ACGAACC
63	CACUGCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGA
74	CCCAGAG CUGAUGAGGCCGAAAGGCCGAA ACGCACU
77	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGACGC
88	GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
101	CUGGAUG CUGAUGAGGCCGAAAGGCCGAA ACAGCGG
105	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGACA
139	UAUUAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
143	UGUUUAU CUGAUGAGGCCGAAAGGCCGAA AGGUACU
146	GACUGUU CUGAUGAGGCCGAAAGGCCGAA AUUAGGU
153	CAGCACU CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
162	CACAAAG CUGAUGAGGCCGAAAGGCCCGAA ACAGCAC
163	GCACAAA CUGAUGAGGCCGAAAGGCCGAA AACAGCA
165	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AGAACAG
166	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAACA
208	UUCAGUG CUGAUGAGGCCGAAAGGCCGAA ACUCUGU
209	UUUCAGU CUGAUGAGGCCGAAAGGCCGAA AACUCUG
227	CGCAAGG CUGAUGAGGCCGAAAGGCCGAA AGGCAUU
228	CCGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGGCAU
231 247	UCACCGC CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
247	GUCUAGG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU
251	UGUCUAG CUGAUGAGGCCGAAAAGGCCGAA AAUUCGC
292	AGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGGAAUU
308	GUCGCAG CUGAUGAGGCCGAAAGGCCGAA AUUUUGUG GAAGCCC CUGAUGAGGCCGAAAGGCCCGAA AGGUUGG
314	GGACCCG CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
315	UGGACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCUA
320	UCUGCUG CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
337	UGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
353	AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
381	UCACUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUGC
407	GGUGCAG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
418	CGAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCGGUG
424	GCCGGGC CUGAUGAGGCCGAAAGGCCGAA AGCAUGA
433	GACCCCA CUGAUGAGGCCGAAAGGCCGAA AGCCGGG
434	UGACCCC CUGAUGAGGCCGAAAGGCCGAA AAGCCGG

440	UCUGCUU CUGAUGAGGCCGAAAGGCCGAA ACCCCAA
449	CUGUAGC CUGAUGAGGCCGAAAGGCCGAA AUCUGCU
453	ACCCCUG CUGAUGAGGCCGAAAGGCCCGAA AGCAAUC
461	UAUCAGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUG
462	GUAUCAG CUGAUGAGGCCGAAAGGCCGAA AACCCCU
463	GGUAUCA CUGAUGAGGCCGAAAAGGCCGAA AAACCCC
468	CAGAUGG CUGAUGAGGCCGAAAGGCCGAA AUCAGAA
473	GCUCGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUAU
491	AGAAGCC CUGAUGAGGCCGAAAAGCCCGAA ACUGGGC
496	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
497	UGGAGAA CUGAUGAGGCCGAAAAGGCCGAA AAGCCGAC
499	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
500	CAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
502	CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
511	AGCAGAU CUGAUGAGGCCGAAAGGCCGAA ACACAUU
514	GAAAGCA CUGAUGAGGCCGAAAGGCCGAA AUGACAC
519	UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AGCAGAU
520	UUUUUUG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
521	AUUUUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
531	CAAGGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
537	CUUGUCC CUGAUGAGGCCGAAAGGCCGAA AGGGUGA
566	GUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
599	CACAGAC CUGAUGAGGCCGAAAGGCCGAA ACAUCAG
· 602	GACCACA CUGAUGAGGCCGAAAGGCCGAA ACAACAU
609	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACCACAG
618	CUCAGCC CUGAUGAGGCCGAAAGGCCGAA ACCACAG
641	UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AUCACCA
867	CGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
0ذ،	UCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUGG
652	GAUCCCG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
653	GGAUCCC CUGAUGAGGCCGAAAAGGCCGAA AAGAUGA
659	CAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCGA
664	GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAU
665	GGAUGGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
671	CCAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGGCAA
674	GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUGG
676	CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
686	UGAUAAA CUGAUGAGGCCGAAAGGCCCGAA ACCAGCA
688	UUUGAUA CUGAUGAGGCCGAAAGGCCGAA AGACCAG
689	UUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAGACCA
690	UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACC
692	CCUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAAAGA
720	GGGGCCU CUGAUGAGGCCGAAAGGCCGAA AUUGGUU
755	GAAAAUU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
759	UCGGGAA CUGAUGAGGCCGAAAGGCCGAA AITHEATE
760	GUCGGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
761	CGUCGGG CUGAUGAGGCCGAAAGGCCCGAA AAAUUGA
762	UCGUCGG CUGAUGAGGCCGAAAGGCCGAA AAAAUUG
771	CCAGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGUCG
	THE PERSON NOUTHER

UCUGGCC CUGAUGAGGCCGAAAGGCCGAA ACUGCCU

CUGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCUCCC

UGGCACU CUGAUGAGGCCGAAAGGCCGAA ACUGGGC

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981 990

Table BXII: Mouse CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
18	GGUgucU u UGCCUCg	479	CAUCACU U UUCgaaa
18	GGuguCU u UGCCucG	480	AUCacuu u ucgaaaa
24	Unugeeu e geeneug	481	UCacuUU U CGAAAAg
38	GCGcgCU a UGGGGCU	481	UCACUUU U cGAAAAG
62	CageGGU c CaUCUag	492	AAAgUGU u AuCCcUG
62	CaGCgGU C CAUCUAG	560	CUAAUGU c aUCUGUG
66	gGUCCAU C uAGggCa	563	AUGUCAU C UGUGGUu
80	AGUGuGU u acgUGca	572	gUGGULU a AagUCcC
80	AgUGUGU u AcgUGCa	572	Gugguuu a aaguccc
81	gUGugUU a CgUGCaG	577	Unaagu c CCgGaug
100	AAACAGU A CCUccac	620	UGGGCAU C CUCAUCA
126	CUGUgaU U UGUGCCA	626	UCCUCAU C AcCaUuu
127	UGUgaUU U GUGCCAG	632	uCAcCAU u UUCGGGg
170 208	CAgcUcU u gaGAaGA	632	UcaCCAU u uUCggGG
208	ggcgaau u Cucagec	634	Accaulu u ccccaug
209	GCGAAUU C UCAGCCC	635	CCaVuuV c GgGGUGu
233 267	gGGAGAU u cgcUgUC	635	cCAULUU C GGGgUgu
267	ACCCAAU c AAggGcu	635	CCAUuuU C ggGGUGu
275	Acccaau c AaGggCu	647	UGUUUCU C UAUAUCA
275	aAGGGCU U CGGGUua	649	ulucucu a UAUCAAA
276	AzGGGcU U CgGgUua	651	ucucuau a ucaaaaa
281	ACCUL C GGGUUAA	653	UCUAUAU C AAAAAGG
281	UUCGGGU u aAGaAGg	735	gGAaGAU u aUCCcGG
314	UUcGGGU u AAGaAGg	759	CGCUGCU C CAGUGCA
354	ACACUGU C UGUACCU	794	Agccugu c Acacagg
386	caAgGaU u GCgaGGC	794	AGcCuGU c acaCAGg
394	cCugUaU c CCUGGCU CCUgGCU u uGGaGuu	819	AGAGAGU C GCAUCUC
394	COUGGCU U UGGaGUU	824	GUCGCAU C UCAGUGC
395	Cuggetu u ggaguua	826	CGCAUCU C AGUGCAG
429	Cacugau a cogucug	876	cccuggu c UgAaCcC
434	AUACCOU C UGUCAUC	913	GGCUGCU U GCUGACC
434	AUACCGU C UGUCAUC	997.	CUCAACU u GCuuUuu
441	Cugticati C Cougcoc	1003	uUGCUUU u uAAggAU
452	CCCCYCA C CCARCEC	1003	uugCUUU u uAaGGAU
452	GCCCAGU C GGCUUCU	1023	gallactu c OGGCaUC
457	פטכפפכט ע כטטכטפפ	1048 1052	CAGUGAU a UCUACCA
458	UCGGCUU C UUCUCCA	. –	gAUauCU a CCaaGuG
460	GGCUUCU U CUCCAAU	1081	CCAGagu u GuCUugc
461	GCUUCUU C UCCAAUC	1084	ayennen c machec
463	UUCUUCU C CAAUcaG	1086	annanch a Concode
472	AAuCAGU C AucaCUu	1097	acagean a cycaany
472	AAUcagU c auCACuU	1098	CGGCGUU C ACUGUAA
		1118	cgUgGCU % CAGGaGU

1118	CgUGGCU a CAggAgU
1141	CgCaGCU u gUGCUCG
1164	accuggu u gccauca
1202	UGuaaUU a UUUaUaC
1220	gGcAuCU c AgAAACu
1220	GGCAUCU C AGAAACu
1228	aGAaACU c UAgcaGG
1253	AaCaGGU a GUGGAAu
1331	AGGAGCU U GCUGCCC
1362	-
	uUuUGaU C CCugGGA
1373	gGGaCUU c AUgguAA
1373 .	GgGACUU c AugguaA
1413	uUGUCAU u DGaccUC
1443	GUaaUGU a CcccGUG
1470 .	CACAUAU c CUaaaAu
1492	GugGUGU a uUGuAga
1497	GUALUGU A gaAaUuA
1508	auUauUU a aUCcGCC
1508	AUUAUUU a auCCGcC
1523	Checkly in Checkly

Table BXIII: Mouse CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
24	CACAGOC CUGAUGAGGCCGAAAGGCCGAA AGGCAAA
38	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGCCCCC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTIC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCCCTIC
66	UGCCCUA CUGAUGAGGCCGAAAGGCCGAA AUGGACC
80	UGCACGU CUGAUGAGGCCGAAAGGCCCGAA ACACACTI
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACTI
81	CUGCACG CUGAUGAGGCCGAAAGGCCGAA AACACAC
100	GUGGAGG CUGAUGAGGCCGAAAGGCCCGAA ACTICITUTE
126	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AUCACAG
127	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AATICACA
170	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAGCTC
208	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AUTICCCC
209	GGGCUGA CUGAUGAGGCCGAAAAGGCCCGAA AAITIYCCC
233	GACAGCG CUGAUGAGGCCGAAAGGCCGAA AUCTICCC
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUTIGCTI
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUTIGGGT
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTTI
275	UNACCCG CHARUGAGGCCGAAAGGCCGAA AGCCTITI
276	UUAACCC GAUGAGGCCGAAAGGCCGAA AAGCCCTI
281	CCUUCUU CUGAUGAGGCCGAAAGGCCCGAA ACCCCAA
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCCAA
314	AGGUACA CUGAUGAGGCCGAAAGGCCGAA ACAGTET
354	GCCUCGC CUGAUGAGGCCGAAAGGCCGAA AUCCERTS
386	AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACAGC
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGA
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
395	UAACUCC CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
429	CAGACGG CUGAUGAGGCCGAAAGGCCGAA AUCACTO
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACCETATI
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACCCTRU
441	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AIRCACTAC
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACTROSS
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACTICCCC
457 · 458	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
460	UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
461	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
	GAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAACC
463	CUGAUUG CUGAUGAGGCCGAAAGGCCCGAA AGAAGAA
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACTEST
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAIX

479	UUUCGAA CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
480	UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AAGIRAII
481	CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAACIIGA
481	CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGIGA
492	CAGGGAU CUGAUGAGGCCGAAAGGCCGAA ACACUUU
560	CACAGAU CUGAUGAGGCCGAAAGGCCGAA ACAUTUAG
563	AACCACA CUGAUGAGGCCGAAAGGCCGAA AUGACAU
572	GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
572	GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
577	CAUCOGG CUGAUGAGGCCGAAAGGCCGAA ACUTUTAA
620	UGAUGAG CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
626	AAAUGGU CUGAUGAGGCCGAAAGGCCCGAA AUGAGGA
632	CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
632	CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
634	. CACCCCG CUGAUGAGGCCGAAAAGGCCGAA AAAUGGU
635	ACACCCC CUGAUGAGGCCGAAAGGCCCGAA AAAAIKG
635	ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
635	ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
647	UGAUAUA CUGAUGAGGCCGAAAGGCCCGAA AGAAACA
649	UUUGAUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
651	UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
653	CCUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAUAGA
735	CCGGGAU CUGAUGAGGCCGAAAGGCCGAA AUCUUCC
759 794	UGCACUG CUGAUGAGGCCGAAAGGCCGAA ACCACCC
794 794	CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
794 819	CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
824	GAGAUGC CUGAUG: "CGAAAGGCCGAA ACUCUCU
826	GCACUGA CUGAUX CCGAAAGGCCGAA AUGCGAC
876	CUGCACU CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
913	GGGUUCA CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
997	GGUCAGC CUGAUGAGGCCGAAAGGCCCGAA AGCAGCC
1003	AAAAAGC CUGAUGAGGCCGAAAAGGCCGAA AGUUGAG
1003	AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1023	AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1048	GAUGCCC CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
1052	UGGUAGA CUGAUGAGGCCGAAAGGCCGAA AUCACUG
1081	CACUUGG CUGAUGAGGCCGAAAGGCCGAA AGAUAUC
1084	GCAAGAC CUGAUGAGGCCGAAAGGCCCGAA ACUCUGG
1086	GCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUC
1097	CCGCAGC CUGAUGAGGCCGAAAGGCCGAA AGACAAC
1098	UACAGUG CUGAUGAGGCCGAAAGGCCGAA ACGCCGC
1118	UUACAGU CUGAUGAGGCCGAAAGGCCGAA AACGCCG
1118	ACTICCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG
1141	ACUCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG CGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGCUGCG
1164	UGALICIC CITCALICACCOCCA AGCUGCG
1202	UGAUGGC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
1220	GUAUAAA CUGAUGAGGCCGAAAAGGCCGAA AAUUACA
1220	AGUUUCU CUGAUGACGCCGAAAAGGCCGAA AGAUCCC
- J -	AGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGCC

1228	CCUGCUA CUGAUGAGGCCGAAAGGCCGAA AGUUUCU
1253	AUUCCAC CUGAUGAGGCCGAAAGGCCGAA ACCUGUU
1331	GGGCAGC CUGAUGAGGCCGAAAGGCCGAA AGCUCCU
1362	UCCCAGG CUGAUGAGGCCGAAAAGGCCGAA AUCAAAA
1177	
1373	UUACCAU CUGAUGAGGCCGAAAGGCCGAA AAGUCCC
1373	UUACCAU CUGAUGAGGCCGAAAGGCCGAA AAGUCCC
1413	GAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGACAA
1443	CACGGG CUGAUGAGGCCGAAAGGCCGAA ACAUUAC
1470	
14/0	AUUUUAG CUGAUGAGGCCGAAAGGCCGAA AUAUGUG
1492	UCUACAA CUGAUGAGGCCGAAAGGCCGAA ACACCAC
1407	
1497	UAAUUUC CUGAUGAGGCCGAAAGGCCGAA ACAAUAC
1508	GGCGGAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAU
1500	
1508	GGCGGAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAU
1523	CAGGUAG CUGAUGAGGCCGAAAGGCCGAA AACCCAG
	CONTRACTOR CONTRACTOR AND CONTRACTOR

Table BXIV: Human B7 Hairpin Ribozyme and Target Sequence

Substrate	GUCAUCA GCC CUGCCUGU CAGCCCU GCU UUGCACCU UCUUUCA GCU CUUGGUGC CACUCU GUU CUGGUGC CACUCU GUU CAGGUGU UGAUCU GUU CAGGUGU UGAUCU GUU CAGGUGU UGAGCU GAC CUUCAAUGU AGAACCO GAC CAUCUUUG CCCAUCU GAC UUCACUUUG CCCAACA GCU UCCCUACA AACCACA GCU UCAUCUGU UGAAUCA GCU UCAUCUGU UGAAUCA GCU UCAUCUGU UGAAUCA GCU UCAUCUGU UGAAUCA GCU CCAUCCU AUANGCU GCC CCAUCCU AUANGCU GCU CCAUCCU AUANGCU GCU UCACCUAC AUAAGCU GCU UUCACCOC AUAAGCU GCU UUCACCCOC AUAAGCU GCU UUCACCCOC AUAAGCU GCU UUCACCCOC AUAAGCU GCU UUCACCCOC	UCUTUCA GAU UAAGCUGA
Hairpin Ribozyme Sequence	ACAGGCAG AGAA GAUGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGAA GGGCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGUCCAA AGAA GGCCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGUCCAA AGAA GAAAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACACCUG AGAA GAAGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACACCUCA AGAA GAAGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA ACCACUA AGAA GAAACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA ACAUCAUA AGAA GAUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GUAGCAA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUUUCAAA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUUUCAAA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUUUCAAA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGAA GUUNAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGCAGUAGA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGCAGUAGA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGCAGUAA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGCAGUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA AGCAGUAA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA ACCAGUAGUACACCCUUGUGGUACAUUACCUCGUA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA AGAA GCUUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA ACCAGUACAACCACCGUUGUGGUACAUUACCUCGUA ACCAGUACAACCACCACACGUUGUGGUACAUUACCUCGUA ACCAGUACACCACACC	OLANGON ALAM GAMAGA ACCAGAGAAACACACGUUGUGGAACAUUACCUGGUA
Наі	GAA GAUGA GAA GAGGA GAA GAGGA GAA GAAGG GAA GAAGGA AA GGUAL	AA GAAAGA
	ACAGGCAG AG GCAAAACA AG GCACCAAG AG GCACCAAG AG GACCACUC AG GACCACUC AG GACCACUC AG ACAUCAUA AG UCUGGGA AG ACAUGAAG AG ACACAUGA AG ACACAUGA AG ACACAUGA AG ACACAUGA AG ACACAUAG AG ACCACUCA AG ACCACUCA AG ACCACUCA AG ACCACUCA AG ACCACUCA AG ACCACUCA AG ACCACUA AG ACCACUCA	- WALL WALL WALL WALL WALL WALL WALL WAL
nt. Position	286 291 295 437 469 518 540 596 644 702 702 702 702 1103 11159 11163 11171 1159	

Table BXV: Mouse B7 Hairpin Ribozyme and Target Sequence

	•	
Substrate	ACACUCU GUI AUDGUCA GUI AUDGUCA GUI UNGUCCU GAU UNGUCCU GAU UNGUCCU GAU UNGUCCU GAU UNGUCCU GAU UNGUCCU GAU CACACU GAC ACAACU GCC ACAAGCU GCC ACAAGCU GUC ACAACCU GUC ACAACCU GUC ACAACCU GUC ACAACCU GUC CUUCUCU GUC	מייירים מרך בממחחרת
Hairpin Ribozyme Sequence	AGAANUGS AGAA GAGUGU ACCAGAGAAACACGGUUGUGGUACAUUACCUGGUA AUCCACCC AGAA GAUGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AAUCGAGA AGAA GAGAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GAAGAGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AAAGACGA AGAA GCACAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCAUCAAC AGAA GAACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCCAA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCCAA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCCAA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCCAA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCAA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGCACA AGAA GCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGACAA AGAA GCAUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AACGACAA AGAA GCUUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUGACGCAA AGAA GCUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUGACGAA AGAA GCUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUGACGAA AGAA GCUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUGACGUA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UANAGGUA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UANAGGUA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UANAGGUA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UANAGGUA AGAA GUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCCGAAAG AGAA GUCUGU ACCAGAGAAACACACGUUGUGGGUACAUUACCUGGUA UCCGAAAG AGAA GCUACA ACCAGAGAAACCACGUUGUGGGUACAUUACCUGGUA UCCGAAAG AGAA GCUCU ACCAGAGAAACACCGUUGUGGCUACAUUACCUGGUA UCCGAAAG AGAA GCUCCU ACCAGAGAAACCACGUUGUGGCUACAUUACCUGGUA CCCACAUG AGAA GCCUC ACCAGAGAAACCACGUUGUGGCUACAUUACCUGGUA CCCACAUG AGAA GCCUC ACCAGAGAAACCACGUUGUGGCUACAUUACCUGGUA CCCACAUG AGAA GCCUC ACCAGAGAAACCACGUUGUGGCUACAUUACCUGGUA CCCACAUGA AGAA GCCUCACAGAAACCACGUUGUGGCUACAUUACCUGGUA CCCACAUGACACACACACGUUGUGCUACAUUACCUGGUA CCCACAUGACCACACACACACCCUCGUACACACCCUCGUACACACCCUCGUACACACCCUCGUACACACCCUCGUACACACCCUCGUACACACCCUCGUACACACCCUCGCOCACACACACACACACACACCCCUCGUACACACACACACAC	
nt. Position	74 114 154 265 328 331 356 373 403 403 403 403 403 1028 1077 1116 1116 1115 1116 1115 1115	

Substrate

HP Ribozyme Sequences

Table BXVI: Human B7-2 Hairpin Ribozyme and Target Sequences

OSITIONII I						
52	GUGACAGO	AGA	GAGAAG	GUINCAGO AGAA GAGAAG ACCAGAGAAACACACGUIGUGGUACAUUACCUGGUA	CUUCUCU GCU GCUGUAAC	AAC AAC
58	CCUGUUAC	AGAA	OCAGAG	GCAGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCUGCU GCU GURACAGG	10c
23	CCCCACUC	AGAA	വേവവ	GUGUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACACACG GAU GAGUGGGG	8
162	CACCAGAG	AGAA	GCAAGC	CACCAGAG AGAA GGAAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ccunccu ecu cucueeue	25
175	UUCAGAGG AGAA	AGAA	5050	GCACCA ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	UGGUGCU GCU CCUCUGAA	A.A.
214	CAUGGCAG	AGNA		GCAGUC ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GACTIGCA GAC CUGCCAUG	AUG
380	CAGGGUCC	AGAA	SUCCES	GUCCGA ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	UCGGACA GUU GGACCCUG	22
408	UGUCCUUG	AGA	GAAGAU	GRAGAU ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	AUCTUCA GAU CAAGGACA	ర్జ
480	CAGAAUUC	AGAA	GCUCGA	GGUGGA ACCAGAGAAACACACGGUGUGUGGUACAUUACCUGGUA	UCCACCA GAU GAAUUCUG	500
575	UAUAGAUG	AGAA	GOUCAA	AGAA GGUCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UDGACCU GCU CAUCUAUA	KU)
710	AACAGACA	AGAA	GAUGGA	AGAA GAUGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCAUCA GCU UGUCUGUU	25
718	GGGAAUGA	AGAA	GACAAG	AGAA GACAAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUUGUCU GUU UCAUUCCC	g
730	CUCCUAAC		GGGAAU	AGAA GGGAAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUTOCCU GAU GUUACGAG	and and
783	AAGAUAAA	AGAA	മാവാ	GCGUCU ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	AGACGCG GCU UUUAUCUU	35
825	CUGGGGGA	AGAA	anoceu	CUGGGGA AGAA QAGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCCUCA GCC UCCCCCAG	3AG
835	GGAAUGUG	AGAA	GGGGGA	AGAA GGGGA ACCAGAAAAACACACGUUGUGGUACAUUACCUGGUA	UCCCCCA GAC CACAUUCC	ပ္ထ
926	GGAAGUAC		GUAAUC	AGAA GUAAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUUACA GCU GUACUUCC	ပ္ထ
968	UAGAAUUA	AGAA	GAMANC	UAGAAUUA AGAA GAAAAC ACCAGAGAAACACACTTTGUGGUACAUUACCUGGUA	GUUUUCU GUC UAAUUCUA	T.
930	AGUUGCGA	AGAA	Canaca	NOUNCEA AGAA GEUUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGAAGCG GCC UCGCAACU	Ş
987	UTUTOUTO	AGAA	GUICAC	UUUUCUUG AGAA GUUCAC ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GUGAACA GAC CAAGAAAA	
1027	UGGGCUUC	AGAA	GAUCTU	UGGGCUUC AGAA GAUCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGAUCU GAU GAAGCCCA	Ą

Table BXVII: Mouse B7.2 Hairpin Ribozyme and Target Sequences

Substrate	ACCURAGE ACTOR CONTINUES		האוליהה האר	ACAACCA GAC	GACCCCA GAU	UGUGACA GUC	ACCUGGUA UCUUGCU GAU CUCAGAUG	ACCUGGUA GAUCUCA GAU GCUGUUUC	ACCUBGUA AGAUGCU GUU UCCGURGA	·	AUGUICA GALI	•					ACCUGGUA GAUUACA GCU UCAGUUAC	ACCUGGUA UGAUGCU GCU CAUCAUUG	_				ACCUGGUA UUCUACA GUU GAAUAAUU	
HP Ribozyme Sequences	UCTUACCC AGAA GCTUGC ACCAGAGAAACACACGUUGUGGUACAUIJACCI KAGIJA	JUGUCAA AGAA GIXETIG ACCAGAGAAACACACTINGICIII CAIGAIA	CURCACISA AGAA CATINGS ACACIONANACION CONTINUISCON CONTIN	CALINATION AGAIN COOKER ACCIONATION CALGADOROGICAL CONTRACTOR CONT	ACCAMANA ACACACOU GO GO GO ACAOU	SCART ACCHARAMACACCOORDINGS ON THE STATE OF	SCANIA ACCAGAGAACACGUGGUGGUACAUUACCUGGUA	LAGAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCAUCU ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GAUAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUNCUG ACCAGAGAAACACACGUUGUGGUACAUIJACCI 19513	AAGAGAGA AGAA GUUGGA ACCAGAGAAACACACGIIIKGIIGGIIACAIIIIACCIICAIII	CACACACC AGAA GOGAAU ACCAGAGAACACACATIIIGIAGIIACAIIIACAICIIA	ACACACAC AGAA GICAIIA ACCACAGAAACACAACIIIGIOCIIIACAIIIACAICA	ACTOR	CONTROL OF THE CONTROL ACCACACACACCOUGUGGGGGACACACGUGGGGAA	CARUCAUS AGAA GCAUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCUGCUA AGAA GAUUCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AACTUAGA AGAA GUGUUG ACCAGAGAAACACACHIIGHGGHACAHIIACGHA	UUCCAAUC AGAA GAGAAC ACCAGAGAAACACAIIIGIIGIIAGIIACIIIA	GANUUCCA AGAA GCUGAG ACCAGAGAACACAGIIICIICIICIIACAIIIACAICIIA	AANUANUC AGAA GUAGAA ACCAGAGAAAACACACGUUGUGGUACAUUACCUGGUA	
Ì	A GCUUGC	A GROCIE	CELLICE					THE CHICAGO.	CCAUCU	GAUAUG	GAACAU	GUUCUG.	GUCCA	GGGAAU	GUCAUA	OT 14 ALE	20000	5005	GAUUCG	GUGGUC	CACAAC	CUCAG	GUAGAA	
	AGA	AGN	ACA	e c				ALPRA I	AGA	AGA	Ž	ACAN	AG A	AGA	AC A	46.04		AGA	A S	AGA	AS A	AGA	AGNA	
	UCTURCE	UUGUCAA	CUACAGGA	CAIRGING	Alicacta acas	CAIMING ACAB		35755	OCCACOGN	AUGGGCAC	UGUCCUUG AGAA	AGAUACUG AGAA	AAGAGAGA	CACACACC	ACACACAC	GIBACTICA	STORY OF THE PROPERTY OF THE P	CARUCAUG	GCCGGCGA	PACCUAGA	UUCCAAUC	GAAUUCCA	ANTUNIUC	
Position	10	42	26	108	146	154	161	101	/97	211	400	679	969	716	737	839	700	# / O	106	929	1115	1118	1133	

Substrate

Hairpin Ribozyme Sequences

Table BXVIII: Human CD40 Hairpin Ribozyme and Target Sequences

																			٠.				
UGGUCCU GCC GCCUGGUC	UCCUGCC GCC UGGUCUCA	UUCGUCU GCC UCUGCAGU	UGGGGCU GCU UGCUGACC	GCUUGCU GAC CGCUGUCC	GCUGACC GCU GUCCAUCC	GACCGCU GUC CAUCCAGA	CAGUGCU GUU CUUUGUGC	CUGCACC GCU CAUGCUCG	ucecece acu unegeeuc	UCAAGCA GAU UGCUACAG	GAGCCCU GCC CAGUCGGC	cueccea auc eccunciun	ccaduce acu ucuncucc	GUCAUCU GCU UUCGAAAA	CAAGACU GAU GUUGUCUG	AGGAUCG GCU GAGAGCCC	GGAUCCU GUU UGCCAUCC	UNIVICEC GAC GAUCTUCE	CAACACU GCU GCUCCAGU	CACUGCU GCU CCAGUGCA	ACAGGCA GUU GGCCAGAG	UGGUGCU GCU GCUGCAGG	vecuecu ecu ecaegegu
GACCAGGC AGAA GGACCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGNA GCAGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACUGCAGA AGAA GACGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGAA GCCCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	SCACAGCG AGAA GCAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	SCAUGGAC AGAA GUCAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	JCUGGAIUG AGAA GCGGUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	SCACAAAG AGAA GCACUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGAGCAUG AGAA GUGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GACCCCAA AGAA GGGCGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGUAGCA AGAA GCUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGAA GGGCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGAAGCC AGAA GGCCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAGAAGA AGAA GACUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUUUCGAA AGAA GAUGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUCTUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUCCU ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GGAUCC ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA	GGAAAA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA	GUGUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCAGUG ACCAGAGAACACACGUUGUGGUACAUUACCUGGUA	GCCUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCACCA ACCAGAGAAACACAC" : GUGGUACAUUACCUGGUA	GCAGCA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA
GCACCA	GCAGGA	GACGAA	5000	GCAAGC	GUCAGC	acaduc	SCACUG	GUCCAG	GCCCGA	CCUICA	ാവാടാ	GGCCAG	GACTUGG	GAUGAC	GUCCUC	GAUCCO	CCAUCC	GGAAAA			accuan	505	SCAGCA
AGAA	AGAA	Age	AGRA	AGA AGA	AGA	A CA	AGA BA	AGA AGA	AGA B	AGA	ACAA	AGAA	AGA	AGA A	AGA	AGAA	NG.	ACAA	AGAA	AGAA	AGAA	ACAA	AGAA
GACCAGGC	UGAGACCA	ACUGCAGA	GOUCHOCH	GCACAGCG	GGAUGGAC	UCUCCAUG	GCACAAAG	CCAGCAUG	GACCCCAA	CUGUAGCA	GCCGACUG	AAGAAGCC	GGAGAAGA	UUUUCGAA	CAGACAAC AGAA	GGGCUCUC AGAA	CGAUGGCA	CCANCAUC	ACUGGAGC	UCCACUGG		CCTGCAGC	ACCCCUGC
26	29	28	84	91	92	98	159	414	429	445	483	488	492	515	593	619	199	764	788	791	924	946	949

Table BXIX: Mouse CD40 Hairpin Ribozyme and Substrate Sequences

Substrate	UGCCUOG GCU GUGCGCCC UGGGCCU GCU LGUUGACA GACAGCG GUC CAUCUAGG GGAAGCC GAC UGACAAGC CUGCACA GCU CUUCAGAA AUUCUCA GCC CAGUGGAA AGACACU GUC UGUACCUG UGAUACC GUC UGUCCUC CAUCCCU GCC CAGUGGAA AGACACU GCC CAGUCGCC CAUCCCU GCC CAGUCGCC CAGUCCCA GCU UCUUCUU CCAGUCG GCU UCUUCUU CCAGUCG GCU UGAAGCCC GAGCCCU GCU GCACCCCC GAGCCCU GCU GCACCCCC CAUCCCU GCU CACUCACC CAUUCCU GCC GCUCACAC CAUUCCU GCU CACUCACAC CAUUCCU GCU CACUCACAC CAUUCCU GCU UGACACAC UGGAACU GCU UGUCACAC UGGAACU GCU UGUCACAC UGGAACU GCU UGUCACAC GCUUGCU GCU UGUCACAC GCUUGCU GCU UGUCACAC GCUUGCU GCU CUUCACAC GCUUGCU GCU UGUCACAC GCUUGCU GCC CCAGACCC GCUUGCU GCC CAAACCCC GCUUGCU GCC CAAACCCC GCUUGCU GCC CAAACCCC GUUGCU GCC CAAACCCC GUUGCU GCC CAAACCCC GUUGCU GCC CAAACCCC GUUGAAACC GCU UUUAAAAC GCUUGCU GCC CAAACCCC GUUGCU GCC CAAACCCC GUUGCU GCC CUAGCCUC
HP Ribozyme Sequences	GCCCCCA AGAA GACCA ACCAGADAAACACGUUGUGGUACAUUACCUGGUA UGUCAACA AGAA GCCCCA ACCAGADAAACACGUUGUGGUACAUUACCUGGUA CCURGAUG AGAA GCUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCUCAAGA GCACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCUCAAGA GAAA GUGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAGGUACA AGAA GUGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAGGUACA AGAA GUGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGAAGC AGAA GCACAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGAAGC AGAA GCACAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGAAGC AGAA GCACAG ACCAGAGAAAACACACGUUGUGGUACAUUACCUGGUA CCCAUUA AGAA GCACAG ACCAGAGAAAACACACGUUGUGGUACAUUACCUGGUA CCCAUCA AGAA GCACAG ACCAGAGAAAACACACGUUGUGGUACAUUACCUGGUA CCCAUCAC AGAA GCACAG ACCAGAGAAAACACACGUUGUGGUACAUUACCUCGUA CCCAUCAC AGAA GCACAG ACCAGAGAAACACACGUUGUGGUACAUUACCUCGUA ACUCAGC ACAA GCACAGAAAACACACGUUGUGGUACAUUACCUCGUA CCCAUCAC AGAA GCACAG ACCAGAAAACACACGUUGUGGUACAUUACCUCGUA ACUCAGC AGAA GCACAGAAAACACACGUUGUGGUACAUUACCUCGUA ACUCAGC AGAA GCACAGAAAACACACGUUGUGGUACAUUACCUCGUA CCCAUCAC AGAA GCACAGAAAACACACGUUGUCGUACAUUACCUCGUA CCCUCCAAAA GAAA GCACAGAAAACACACGUUGUCGUACAUUACCUCGUA CCCUCCAAA GAAA GCCAGAAAACACACGUUGUCGUACAUUACCUCGUA CCUCCAAAAA GAAA GCCAGAAAAACACACGUUGUCGUACAUUACCUCGUA UCAACAGA AGAA GCCAGAAAAACACACGUUGUCGUACAUUACCUCGUA CCUCCAAAAAG AGAA GCCAGAAAACACACGUUGUCGUACAUUACCUCGUA UCAAAAG AGAA GCCAGAAAACACACGUUGUCGUACAUUACCUCGUA CCUCCAAAAAG AGAA GCCAGAAAAACACACGUUGUCGUACAUUACCUCGUA CCCUCCAAAAAG AGAA GCCAGAAAAACACACGUUGUCGUACAUUACCUCGUA CCCUCCAAAAAAAAAA
nt. Position	25 45 144 164 212 212 311 444 444 444 444 449 453 550 580 592 605 752 752 787 909 909 916 9137 1137 1137

Table CII: 2.5 μ mol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 μL	2.5
S-Ethyl Tetrazole	23.8	238 μL	2.5
Acetic Anhydride	100	233 μL	5 sec
N-Methyl Imidazole	186	233 μL	5 sec
TCA	83.2	1.73 mL	21 sec
lodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

^{*} Wait time does not include contact time during delivery.

Table EVII: Deprotection of a 36 mer all ribo oligo using 70% ethylamine in aqueous. The data are as follows upon HPLC reprocessing:

Sample	OD's	% Full Length Product (FLP)	% frontside	%backside
MA 10'@65°	0.984	14.5073	71.6740	13.8186
MA 10'@65°	1.125	18.9269	67.8006	13.2725
EA rt 10'	0.925	16.5804	66.8186	16.6010
EA rt 10'	0.920	15.7421	67.5794	16.6785
EA rt 30'	0.971	17.4694	67.6782	14.8525
EA rt 30'	0.794	15.7587	69.8084	14.4329
EA 40° 10'	0.819	18.0827	66.4937	15.4236
EA 40° 10'	0.986	17.5763	66.7865	15.6372
EA 40° 15'	0.877	18.7963	67.0064	14.1999
EA 40° 15'	0.911	18.7808	70.7306	10.4885
EA 55° 10'	1.001	17.8810	66.4703	15.6487
EA 55° 10'	1.023	19.1069	68.6706	12.2225

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<u>Claims</u>

- An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.
- The enzymatic nucleic acid of claim 1, wherein said nucleic acid
 comprises a 3'-3' linked inverted ribose moiety at said 3' end.
 - 3. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
 - 4. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
- An enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 6-methyl uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least

ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.

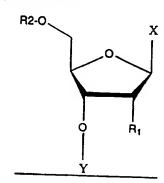
- 6. The enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moiety at its 3' end.
- The enzymatic nucleic acid of any one of claims 1-6, wherein said nucleic acid comprises phosphorothioate linkages at least three of the seven 5' terminal nucleotides.
 - Nucleic acid molecule which blocks synthesis and/or expression of an mRNA encoding B7-1, B7-2, B7-3 and/or CD40.
 - 9. The nucleic acid of claim 8, wherein said molecule is an enzymatic nucleic acid molecule.
- 20 10. The nucleic acid molecule of claim 9, wherein, the binding arms of said enzymatic nucleic acid contain sequences complementary to the nucleotide base sequences in any one of Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX.
- 11. The nucleic acid molecule of claims 9 or 10, wherein said nucleic acid25 molecule is in a hammerhead motif.
 - The enzymatic nucleic acid molecule of claim 9 or 10, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid or RNaseP nucleic acid motif.

- 13. The enzymatic nucleic acid molecule of any of claims 9 or 10, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.
- 14. The enzymatic nucleic acid of claim 13, wherein said ribozyme
 comprises between 14 and 24 bases complementary to the RNA of said region.
 - 15. Enzymatic nucleic acid molecule consisting essentially of any ribozyme sequence selected from those shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII.
- 16. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 8 or 9.
 - 17. The cell of claim 16, wherein said cell is a human cell.
- 18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 9 or 10, in a manner which allows expression and/or delivery of that enzymatic RNA molecule within a mammalian cell.
 - 19. A mammalian cell including an expression vector of claim 18.
 - 20. The cell of claim 19, wherein said cell is a human cell.
- 21. A method for treatment of a patient having a condition associated with the level of B7-1, B7-2, B7-3 and/or CD40, wherein the patient, tissue donor or population of corresponding cells is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 8, 9 or 10.
- 22. A method for treatment of a condition related to the level of B7-1, B7-2,
 B7-3 and/or CD40 activity by administering to a patient an expression vector of claim 21.
 - 23. The method of claims 21 or 22, wherein said patient is a human.

- 24. A method for inducing tolerance in a recipient to alloantigen of a donor comprising treating antigen presenting cells from a donor with nucleic acid of claim 8 or 9, and infusion of said treated antigen presenting cells into said recipient.
- 5 25. A method for enhancing graft tolerance comprising contacting a nucleic acid of claims 8 or 9 with cells of said graft prior to transplantation.
 - 26. A method for treatment of an autoimmune disease, comprising contacting an antigen presenting cell of a patient with a nucleic acid of claims 8 or 9.
- 10 27. The method of claim 26, wherein said cells are contacted ex vivo with said nucleic acid.
 - The method of claim 26, wherein said cells are contacted with autoantigen characteristic of said disease.
- The method of claim 28, wherein said cells are reinfused into saidpatient.
 - 30. Enzymatic nucleic acid having at least one modified base substitution, wherein said base substitution is selected from a group comprising pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.
 - 31. The enzymatic nucleic acid of any of claim 30, wherein said nucleic acid has a hammerhead motif.
 - 32. Mammalian cell comprising an enzymatic nucleic acid molecule of and of claims 30-31.
- 25 33. The enzymatic nucleic acid of claim 31, wherein said nucleic acid includes said modified base substitutions at position 4 or at position 7.
 - 34. The ribozyme of claim 33, wherein said substitution is 6-methyl uracil.
 - 35. The ribozyme of claim 33, wherein said substitution is pyridin-4-one.

- 36. The ribozyme of claim 33, wherein said substitution is phenyl.
- 37. The ribozyme of claim 33, wherein said substitution is pyridin-2-one.
- 38. The ribozyme of claim 33, wherein said substitution is pseudouracil.
- 39. The ribozyme of claim 33, wherein said substitution is 2, 4, 6-trimethoxy benzene.
 - 40. The ribozyme of claim 33, wherein said substitution is dihydrouracil.
 - 41. The ribozyme of claim 33, wherein said substitution is 3-methyluracil.
 - 42. The ribozyme of claim 33, wherein said substitution is naphthyl.
 - 43. The ribozyme of claim 33, wherein said substitution is aminophenyl.
- 10 44. 2'-deoxy-2'-alkylnucleoside.
 - 45. 2'-deoxy-2'-alkylnucleotide.
 - 46. Oligonucleotide comprising one or more 2'-deoxy-2'-alkylnucleotides.
 - 47. Enzymatic nucleic acid comprising a 2'-deoxy-2'-alkylnucleotide.
- 48. Method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group.
 - 49. 2'-deoxy-2'-alkylnucleotide triphosphate.
- 50. Method for synthesis of a 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base comprising the steps of:
 - (a) phenoxyltriocarbonylation of 5'-O-DMT-3'-O-TBDMS-base to yeild a thioester, replacing a 2' hydroxyl group with a phenoxythiocarbonyl group, and

- (b) Heck acylation of said thioester to form a 2'-C-allyl derivative in which said 2'-phenoxythiocarbonyl group is replaced with said 2'-C-allyl group to yield said 2'-C-allyl derivative.
- 51. A compound having the formula:



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wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents O, DMT or a phosphorus-containing group.

- 52. Oligonucleotide comprising one or more compounds of claim 51.
- 10 53. Enzymatic nucleic acid comprising a compound of claim 51.
 - 54. The compound of claim 51, wherein said compound is in the form of a triphosphate.
 - 55. Enzymatic nucleic acid of claim 53 wherein said nucleic acid is in a hammerhead motif.
- 15 56. Enzymatic nucleic acid of claim 53, wherein said nucleic acid is in a hairpin, hepatitis delta virus, group I intron, VS RNA or RNase P RNA motif.
 - 57 Enzymatic nucleic acid of claim 55, wherein said hammerhead ribozyme has positions 4 and/or 7 substituted with 2'-O-methylthiomethyl.

- 58. Enzymatic nucleic acid of claim 55 or 57, wherein one monomer in stem II of said hammerhead is substituted with at least one 2'-Omethylthiomethyl.
- 59. Enzymatic nucleic acid of claim 55 or 56, wherein said nucleic acid is substituted at one or more positions with 2'-O-methylthiophenyl.
- 60. A mammalian cell comprising a compound of any one of the claims 51-59.
- 61. The cell of claim 60, wherein said cell is a human cell.
- 62. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.
- 64. Hammerhead ribozyme having a non-nucleotide in the catalytic core in a site selected from the group consisting of the normally occurring uracil at position 4 and 7.
 - 65. Hammerhead ribozyme having a stem II and a loop II, wherein said loop II comprises a non-nucleotide.
 - 66. Hammerhead ribozyme having a non-nucleotide at its 3' end.
- 20 67. A mammalian cell comprising an enzymatic nucleic acid molecule of any one of the claims 64-67.
 - 68. The cell of claim 67, wherein said cell is a human cell.
 - 69. Method of synthesis of abasic ribonucleoside mimetics described in figure 58.
- 25 70. A method for the deprotection of RNA comprising the step of providing aqueous ethylamine (EA) at between 25°C 60°C for 5 to 30 minutes to remove any exocyclic amino protecting groups from protected RNA.

- 71. The method of claim 70 wherein, said ethylamine is provided at 40°C for 10 minutes.
- 72. The method of claim 70 wherein, said ethylamine is provided at 55°C for 10 minutes.
- The method of claim 70, further comprising deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 74. The method of any one of claims 70-73 wherein, said RNA is an enzymatic RNA.
 - 75. Method for synthesis of an enzymatic nucleic acid, comprising the steps of:
- providing a 3' and a 5' portion of said enzymatic nucleic acid having independent chemically reactive groups at the 5' and 3' positions, respectively, under conditions in which a covalent bond is formed between said 3' and 5' portions by said chemically reactive groups, said bond being selected from the group consisting of, disulfide, morpholino, amide, ether, thioether, amine, a double bond, sulfonamide, ester, carbonate, hydrazone, said bond not being a natural bond formed between a 5' phosphate group and a 3' hydroxyl group.
 - 76. The method of claim 75, wherein said nucleic acid has a hammerhead motif and said 3' and 5' positions each have said chemically reactive groups in or immediately adjacent to the stem II region.
- 77. The method of claim 75, wherein one said chemically reactive group is (CH₂)_nSH and the other chemically reactive group is (CH₂)_nSH, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 78. The method of claim 75, wherein one said chemically reactive group is (CH₂)_nNH₂ and the other chemically reactive group is ribose, wherein

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each n independently is an integer from 0 to 10 inclusive and may be the same or different.

- 79. The method of claim 75, wherein one said chemically reactive group is (CH₂)nNH₂ and the other chemically reactive group is COOH, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 80. The method of claim 75, wherein one said chemically reactive group is (CH₂)nX and the other chemically reactive group is (CH₂)_nOH or (CH₂)_nSH; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different; X is halogen.
- 81. The method of claim 75, wherein one said chemically reactive group is (CH₂)nNH₂ and the other chemically reactive group is CHO, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 15 82. The method of claim 75, wherein one said chemically reactive group is (CH₂)nPPh₃ and the other chemically reactive group is CHO, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 83. The method of claim 75, wherein one said chemically reactive group is (CH₂)nNH₂ and the other chemically reactive group is (CH₂)nSO₂CI, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
 - 84. The method of claim 75, wherein one said chemically reactive group is (CH₂)nOH and the other chemically reactive group is COOH, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
 - 85. The method of claim 75, wherein one said chemically reactive group is (CH₂)_nCOH and the other chemically reactive group is (CH₂)_nNH₂, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.

- 86. The method of claim 75, wherein one said chemically reactive group is (CH₂)_nCOX and the other chemically reactive group is (CH₂)_nOH, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 5 87. The method of claim 78, wherein said conditions include provision of NaI0₄ in contact with said ribose, and subsequent provision of NaBH₄ or NaCNBH₃.
 - 88. The method of claim 79, wherein said conditions include provision of a coupling reagent.
- 89. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of (CH₂)_nSH, (CH₂)_nNH₂, ribose, COOH, (CH₂)_nX, (CH₂)_nPPh₃, CHO, (CH₂)_nSO₂CI, (CH2)_nCOX, (CH₂)_nX, (CH₂)_nOH, (CH2)_nCOH, and (CH₂)_nSH; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or "fferent and X is halogen.
 - 90. The method of claim 75, wherein one said chemically reactive group is linking group-SH and the other chemically reactive group is linking group-SH, wherein each linking group may be the same or different.
- 20 91. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is ribose.
 - 92. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is COOH.
- 93. The method of claim 75, wherein one said chemically reactive group is linking group-X and the other chemically reactive group is linking group-OH or linking group-SH; wherein each linking group may be the same or different; X is halogen.
 - 94. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is CHO.

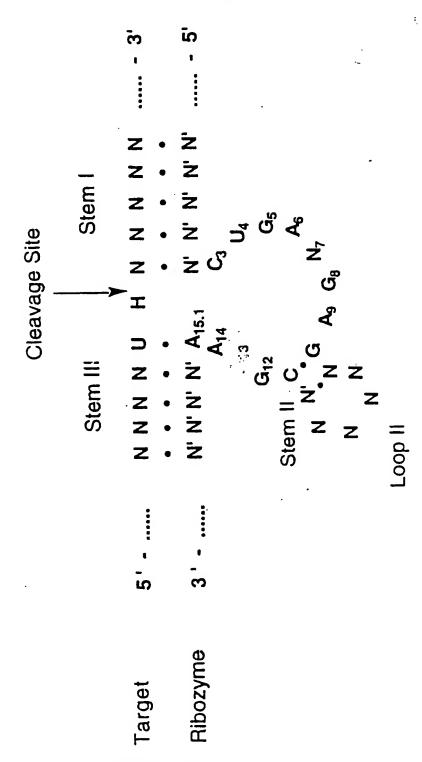
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- 95. The method of claim 75, wherein one said chemically reactive group is linking group-PPh₃ and the other chemically reactive group is CHO.
- 96. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is linking group-SO₂CI, wherein each linking group may be the same or different.
- 97. The method of claim 75, wherein one said chemically reactive group is linking group-OH and the other chemically reactive group is COOH.
- 98. The method of claim 75, wherein one said chemically reactive group is linking group-COH and the other chemically reactive group is linking group-NH2, wherein each linking group may be the same or different.
 - 99. The method of claim 75, wherein one said chemically reactive group is linking group-COX and the other chemically reactive group is linking group-OH, wherein each linking group may be the same or different.
- 15 100. The method c 'iin 91, wherein said conditions include provision of NalO₄ in contact with said ribose, and subsequent provision of NaBH₄ or NaCNBH₃.
 - 101. The method of claim 100, wherein said conditions include provision of a coupling reagent.
- 102. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of linking group-SH, linking group-NH₂, ribose, COOH, linking group-X, linking group-PPh₃, CHO, linking group-SO₂CI, linking group-COX, linking group-X, linking group-OH, linking group-COH, and linking group-SH; wherein each linking group may be the same or different and X is halogen.
 - 103. A transcribed non-naturally occuring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said

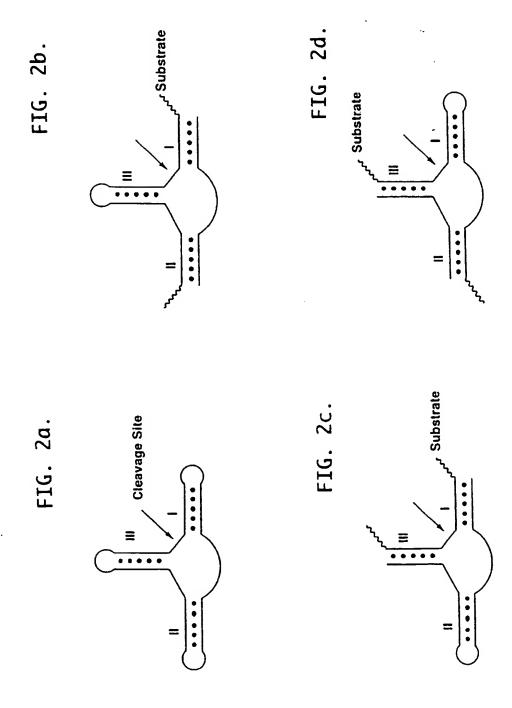
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stem comprises at least 8 base pairs wherein said molecule is transcribed by a RNA polymerase II promoter system.

- 104. A transcribed non-naturally occuring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by a U6 small nuclear RNA promoter system.
- 105. A transcribed non-naturally occuring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by an adenovirus VA1 RNA promoter system.
- 15 106. A transcribed non-naturally occuring RNA molecule, comprising a desired therapeutic F, ''. portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is a chimeric adenovirus VA1 RNA.
 - 107. A transcribed non-naturally occuring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said intramolecular stem is separated from said desired RNA by a spacer sequence.
 - 108. The RNA molecule of claim 107, wherein said spacer sequence is about 5-50 nucleotides.

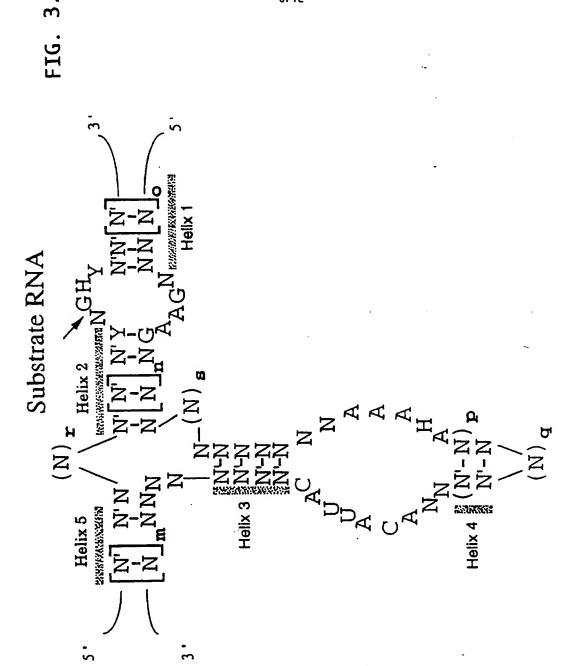


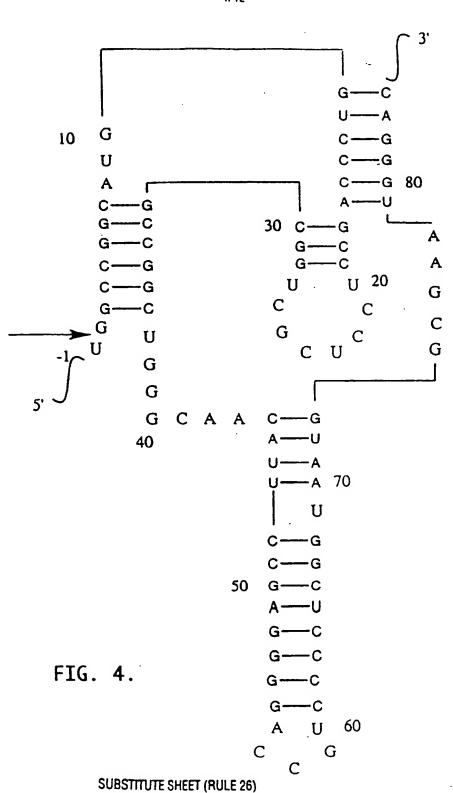
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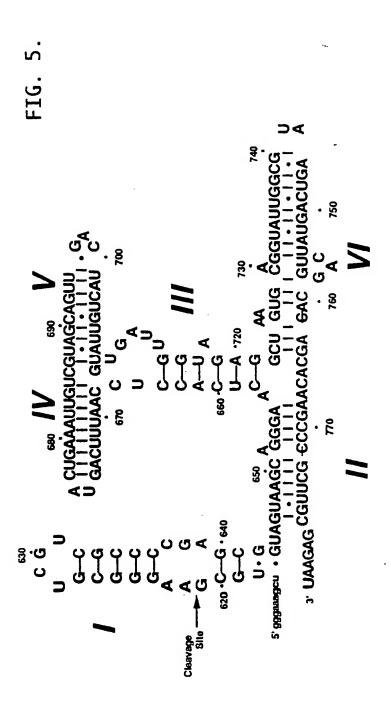


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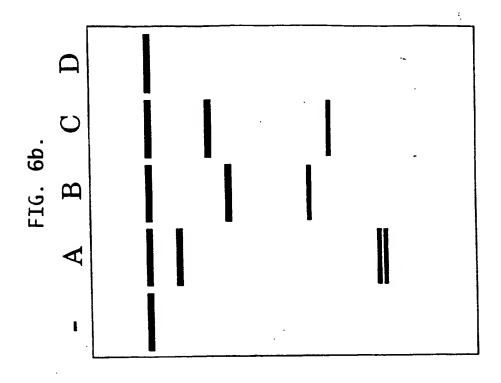






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FIG. 6a.



•Body-labeled transcript
(not purified)
•DNA oligo
(10 nM, 100 nM and 1000 nM)
•RNAse H
(0.08 -1.0 u/µl)

•37°C, 10 min

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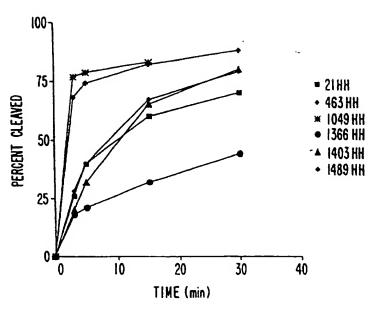
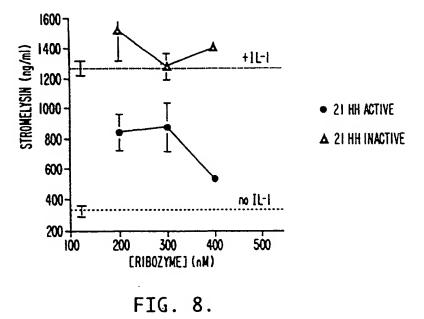


FIG. 7.



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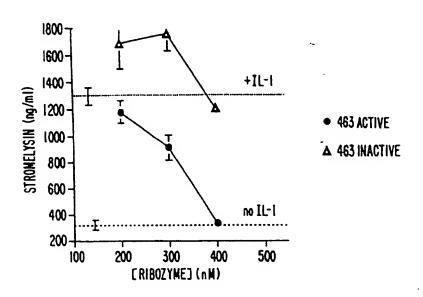


FIG. 9.

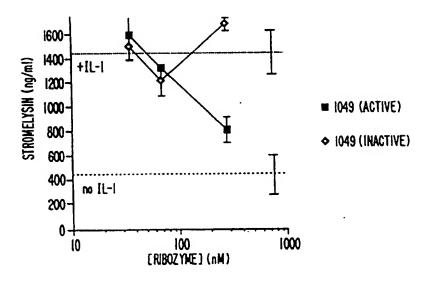
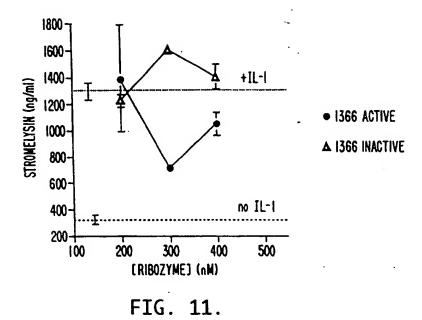


FIG. 10.

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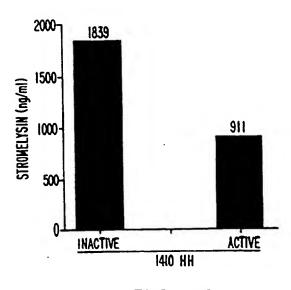


FIG. 12.

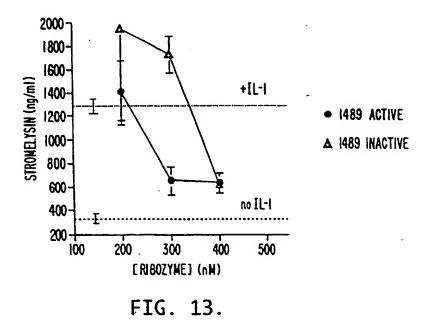


FIG. 14.
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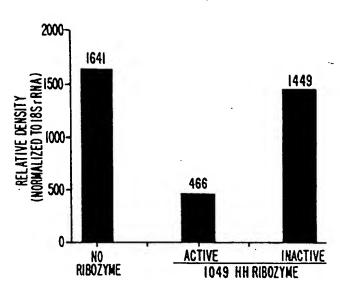


FIG. 15.

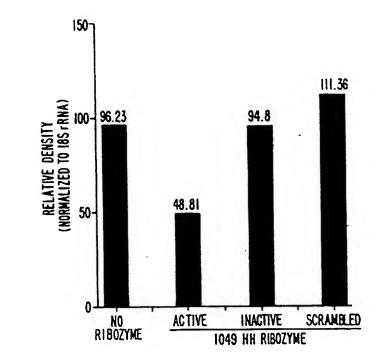
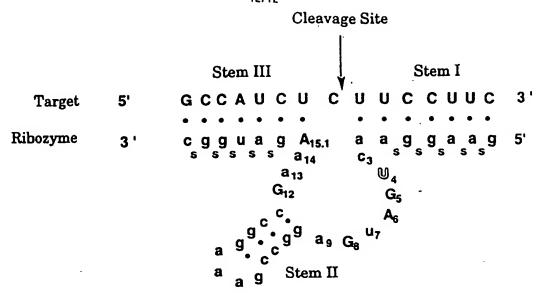


FIG. 16.



Upper case= ribonucleotides
Lower case= 2'-O-methyl nucleotides

U = 2'-C-Allyl modification

s = phosphorothioate linkages

FIG. 17a.

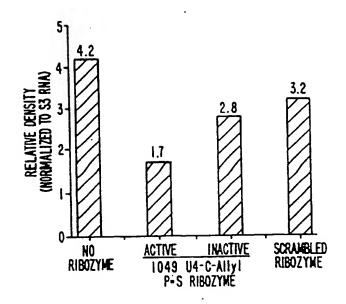
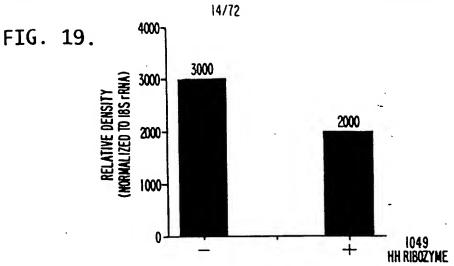
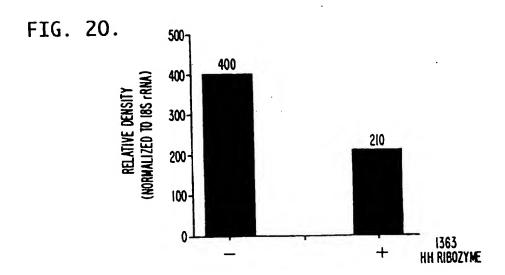


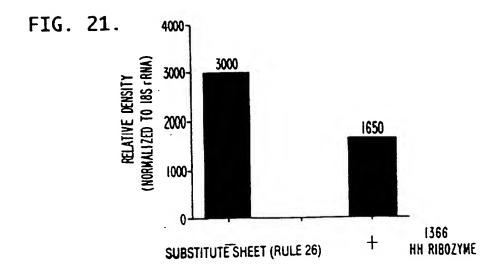
FIG. 17b. SUBSTITUTE SHEET (RULE 26)

```
UUCCUUC --- 3'
5'--- G C C A U C U C
        ggua
                                                  FIG. 18a.
                  a
                            G
                               UPPER CASE=ribonucleotides
                  G
                               LOWER CASE=2'-0-Methylnucleotides
                                U=2'-amino
                                s=phosphorothioatelinkages
             a
                 g
                         1049 2'-AMINO P=S RIBOZYME
 5'--- AUGCUGUU
                        UUUGAAG--- 3'
                          a a
                          C
                                                  FIG. 18b.
                   a
                            G
                                UPPER CASE=ribonucleotides
                  G
                                LOWER CASE=2'-0-Methylnucleotides
                                U=2'-amino
                                s=phosphorothioatelinkages
                         1363 2'-AMINO P=S RIBOZYME
                         GAAGAAU --- 3'
 5'--- C U G U U U U
                           c u u
                                                  FIG. 18c.
                    а
                                UPPER CASE=ribonucleotides
                                LOWER CASE=2'-O-Methylnucleotides
                                U=2'-amino
                                s=phosphorothioatelinkages
             а
                         HUMAN 1366 2'-AMINO P=S RIBOZYME
                         GAAGCAU --- 3'
  5'--- C U G U U U U U
          acaaaA
                           C
                                                   FIG. 18d.
                           C
                    a
                   G
                                 UPPER CASE=ribonucleotides
                                 LOWER CASE=2'-O-Methylnucleotides
                                 ü=2'-amino
                                 s=phosphorothioatelinkages
              а
                          RABBIT 1366 2'-AMINO P=S RIBOZYME SUBSTITUTE SHEET (RULE 26)
              а
                  g
                а
```

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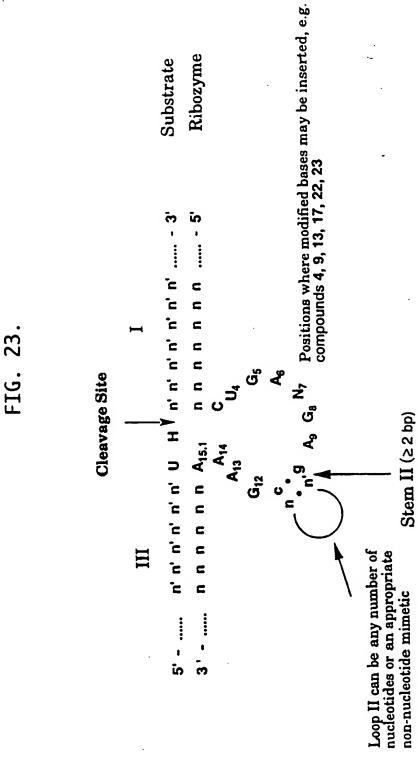


2-Amino-Purine

5-Fluoro-Cytosine

5-Bromo-Cytosine

N⁴,N⁴-dimethyl-Cytosine



SUBSTITUTE SHEET (RULE 26)

5-Halouridine

Diaminopurine

II 8

SUBSTITUTE SHEET (RULE 26)

DMT-CVPyr Ac₂O/Py Triazole, POCI₃/Et₃N/CH₃CN

SUBSTITUTE SHEET (RULE 26)

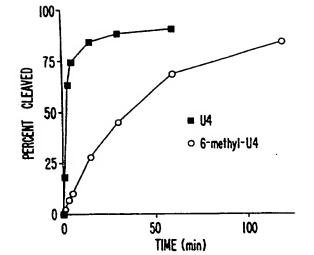
SUBSTITUTE SHEET (RULE 26)

REAGENTS AND CONDITIONS:

- i) 6-Me-Ura^{TMS}, CF₃SO₃SIME₃,0°C;
- ii) 1,2,4-triazole, POCl₃; iii) NH₄OH/dioxane;
- iv) 2M NaOH/Pyr/MeOH; v) MeSI-CL/PYR, THEN AC2O;
- vi) DMT-Cl/Pyr;
- vii) TBDMS-Cl/AgNO₃/Pyr/THF;
- viii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/ CH_2Cl_2 .

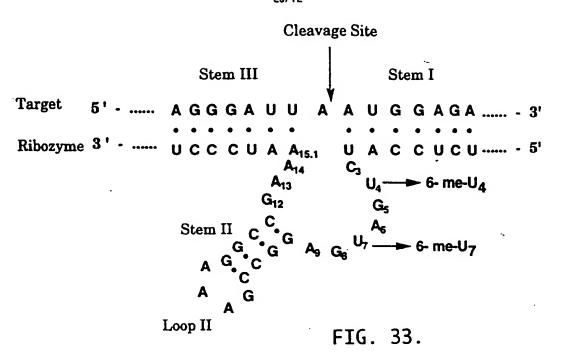
FIG. 30.

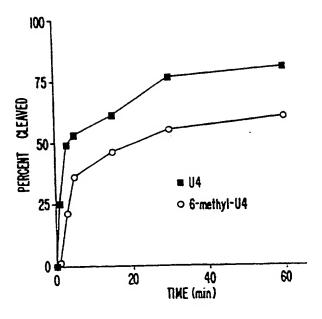
FIG. 31.



[Ribozyme]=40nM [Substrate]=~1nM

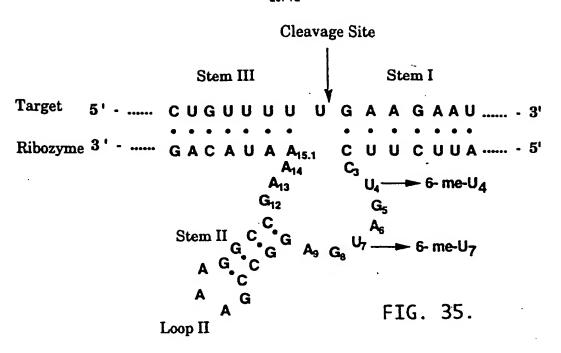
FIG. 32.
SUBSTITUTE SHEET (RULE 26)

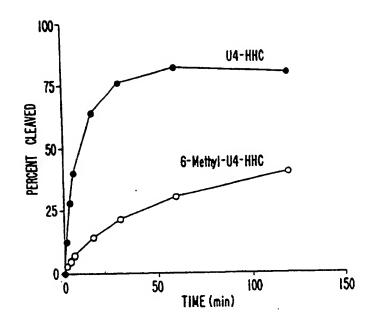




[Ribozyme]=40nM [Substrate]=~1nM

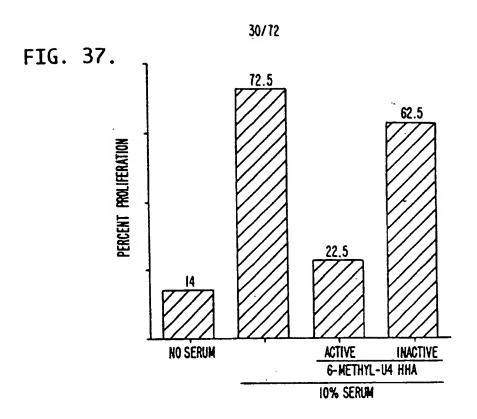
FIG. 34. SUBSTITUTE SHEET (RULE 26)

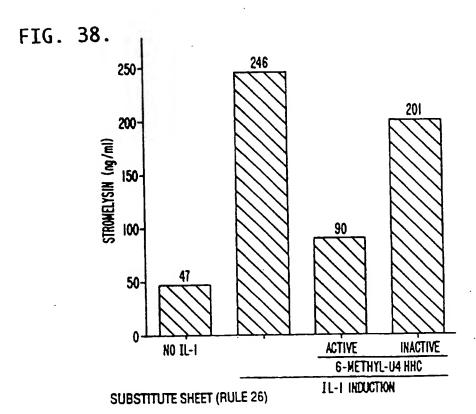




[Ribozyme]=40nM [Substrate]=~1nM

FIG. 36. SUBSTITUTE SHEET (RULE 26)





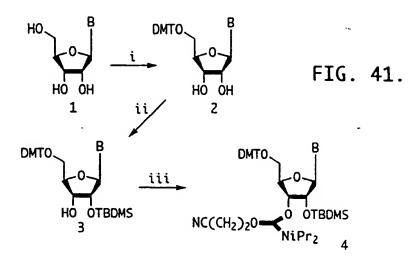
Bz = Benzoyl
Ac = Acetyl
DMT = 4,4'-Dimethoxytrityl
TBDMSi = \(\ellar{c}\)-Butyldimethylsilyl
CE = 2-cyanoethyl

Reagents and Conditions: i: N,O-bis(trimethylsilyl)acetamide (BSA)/TMSTfl/CH₃CN, 70 °C, ii: NaOCH₃/CH₃OH, iii: DMT-Cl/DMAP/Et₃N/Pyr, iv: TBDMSi-Cl/AgNO₃/Pyr/THF, v: P(OCE)(N-iPr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂.

FIG. 39.

Reagents and Conditions: I: PhLi/THF, -78 °C, II: Et3SIH/BF3.Et2O/CH3CN, -40 °C, III: 1M TBAF/THF, Iv: 70 % aq. CH3COO!-3, 100 °C, v: DMT-CI/DMAP/Et3N/Pyr, vi: TBDMSI-CVAgNO3/Pyr/THF, vii: P(OCE)(N-IPr2)CI/DIPEA/1-Molm/CH2CI2.

FIG.



B=Pseudo U,2,4,6-trimethoxy benzene or3-methyl U

REAGENTS AND CONDITIONS:

- i) DMT-Cl/Pyr;
- ii) TBDMS-Cl/AgNO 3/Pyr/THF;
- iii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

FIG. 42.

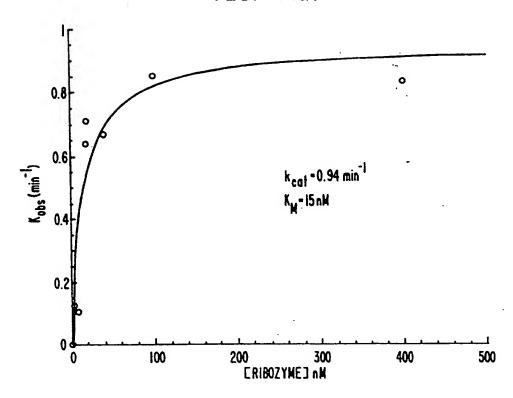
DMT = 4,4'-Dimethoxytrityl TBDMSi = t-Butyldimethylsilyl CE = 2-Cyanoethyl

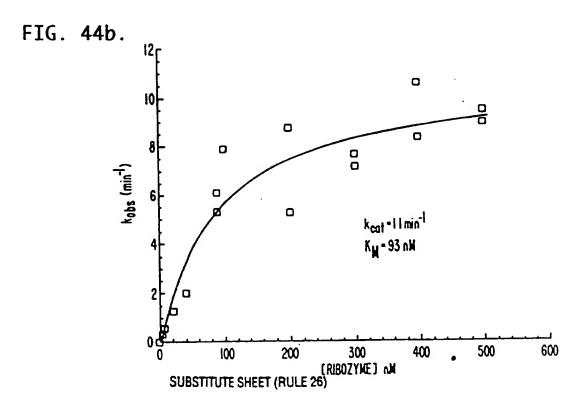
Reagents and Conditions: i: Pd/Rh, H2 60 psi, ii: DMT-CVDMAP/Et3N/Pyr, iii: TBDMSi-CVAgNO3/Pyr/THF, iv: P(OCE)(N-iPr2)CVDIPEA/1-MeIm/CH2Cl2

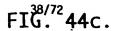
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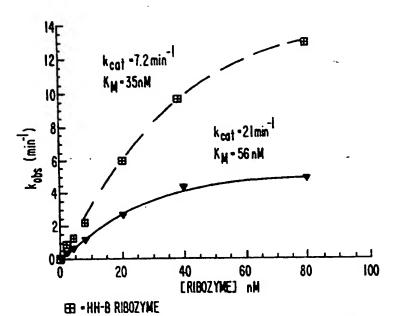
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		4)	
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			•

^{37/72} FIG. 44a.









♥ - HH-B RIBOZYME WITH PHENYL-SUBSTITUTION AT POSITION 7

FIG. 45.

2

TBDPSiO

TBDPSIO

TBDPSIO

TBDPSIQ

NHTFA

TBDPSIQ

TBDPSIQ

DMT = 4,4'-Dimethoxytrity! 6
TBDMSi = t-Butyldimethylsily! 2.Cxanoethyl TBDPSi = t-Butyldiphenylsilyl DMT = 4,4'-Dimethoxytrityl

CE = 2-Cyanoethyl

I O DMTO ×

OTBDMS CEO'P'NPr

3'-isomer

Reagents and Conditions: I: 1-Li-4-bromobenzene/THF, -78 °C, II: Et3SIH/BF3.Et2O/CH3CN, -40 °C, III: Iiq. NH3/Cul, 115 °C, iv: TFA2O/Pyr, v: 1M TBAF/THF, vi: 70% aq. CH3COOH, 100 °C, vii: DMT-CVDMAP/Et3N/Pyr, viii: TBDMSI-CVAgNO3/Pyr/THF, ix: P(OCE)(N-IPP)CVDIPEA/1-MeIm/CH2CI2.

OTBDMSi NHTFA

DMTQ

9 0 DMTQ

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	·	Table 1 Entries	12-14	9-11	3-5	8-9	21-22	15-17	18-20	7
-S-			$U4 \& U7 = 2^{-}C-Allyl-U$	U4 & U7 = 2'-F-ribo-U	U4 & U7 = 2'=CH ₂ -U	$04 \& U7 = 2'=CF_2-U$	U4 & U7 = 2'-dU	U4 & U7 = 2'-F-ara-U	$U4 \& U7 = 2'-NH_2-U$	U4 & U7 = 2'-O-Me-ribo-U
ucccuarA uaccucu	15.1 a c 4	7 7 5 6 5 II	6 ° 6 ° 6	ა თ უ დ	G				Lower case = 2'-O-Me	rv = nbonucleolide
3,-										

FIG. 47.

B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

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SUBSTITUTE SHEET (RULE 25)

Marklewicz reagent v) = DMTCI/Pyr DMSO & Ac₂O vi) = Ph₃P, CICF₂COONa Ph₃PCH₃I vii) = P(OCE)(N-iPr₂)Cl

| (≥

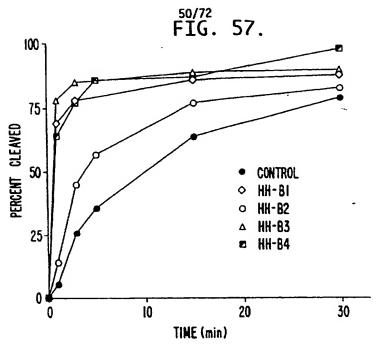
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FIG. 52.

SUBSTITUTE SHEET (RULE 26)

iv) = DMTCVPyr = $P(OCE)(N-iPr_2)Ci$ vi) = 1,2,4-triazole, $P(O)Ci_3$ vii) = 29% $NH_4OH/dioxane$, Ac_2O/Pyr = PhOC(S)CVPy ii) = Allyltributyltin, Bz₂O₂ or AIBN /tolueneiii) = TBAF ?

```
A U G G A G A --- 3'
   - AGGGAUU
                        Uaccucu5'
   3' U C C C U a A
                                            FIG. 56a.
                 a
                               HH-B1
                G
                             UPPER CASE=ribonucleotides
                             LOWER CASE=2'-O-Methyl nucleotides
                             U and C=2'-0-Methylthiomethyl
                            (() =2'-Amino
                      AUGGAGA --- 3'
5'--- A G G G A U U A
                        UACCUCU5'
   3' U C C C U A A
                                            FIG. 56b.
                               HH-B2
                            UPPER CASE=ribonucleotides
                            LOWER CASE=2'-0-Methyl nucleotides
                            U,A,G and C=2'-0-Methylthiomethyl
                            (U) = 2' Amino
                      A U G G A G A --- 3'
5'--- A G G G A U U A
                        Uaccucu5'
   3' UCCCUaA
                                            FIG. 56c.
                  а
                               HH-B3
                             UPPER CASE=ribonucleotides
                             LOWER CASE=2'-0-Methyl nucleotides
                             C=2'-0-Methylthiomethyl
                             U=2'Amino
                      A U G G A G A --- 3'
5'--- A G G G A U U A
                         uaccucu5'
   3' ucccua A
                                             FIG. 56d.
                  а
                                HH-B4
                 G
                              UPPER CASE=ribonucleotides
                              LOWER CASE=2'-O-Methyl nucleotides
                              U=2'-Methylthiomethyl
                       SUBSTITUTE SHEET (RULE 26)
```



Si = t-Butyldimethylsllyl
DMT = 4,4'-Dimethoxytrityl

CE = Cyanoethyl

Reagents and Conditions: i) PhOC(S)-CVDMAP, ii) Bu3SnH/AIBN, iii) CF3COOH, DMT-CVPyr, iv) Bu3SnH/Bz2O2, v) 2M NaOH/Pyr/MeOH, DMT-CVPyr, vi) TBDMS-CVAgNO3, vii) P(OCE)(N-iPr2)CI SUBSTITUTE SHEET (RULE 26)

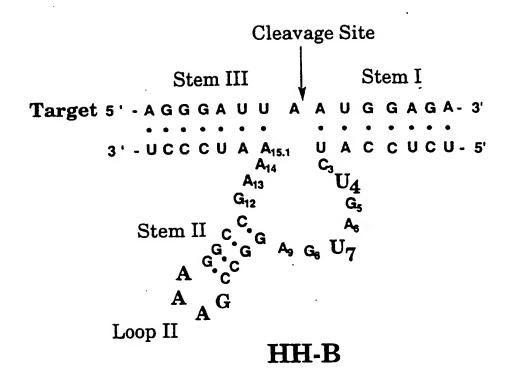


FIG. 59.

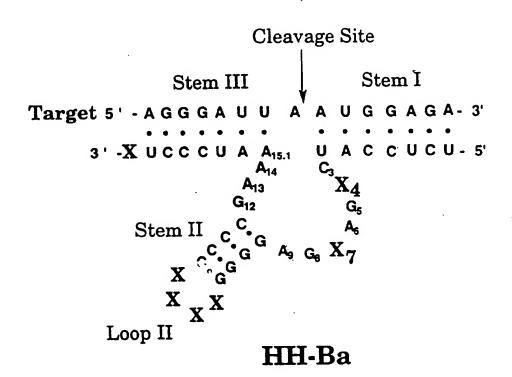


FIG. 60.

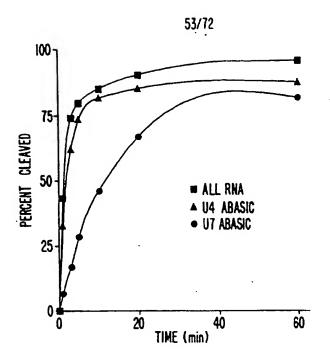


FIG. 61.

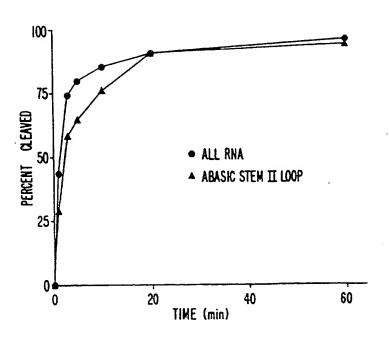
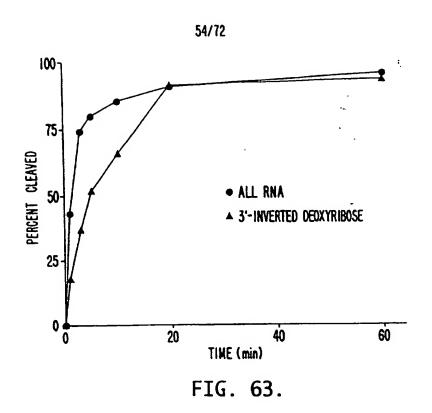


FIG. 62.
SUBSTITUTE SHEET (RULE 26)

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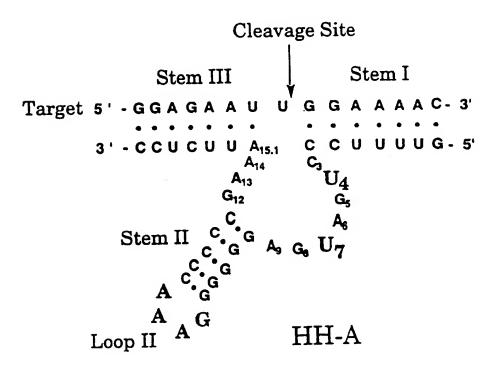


FIG. 64. SUBSTITUTE SHEET (RULE 26)

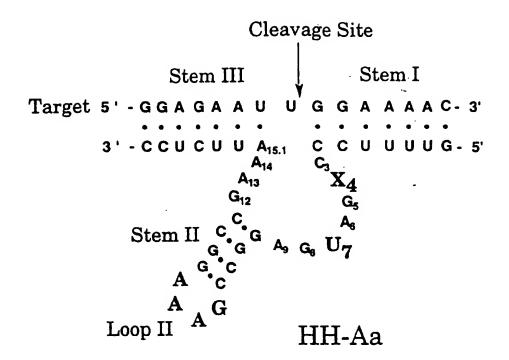


FIG. 65.

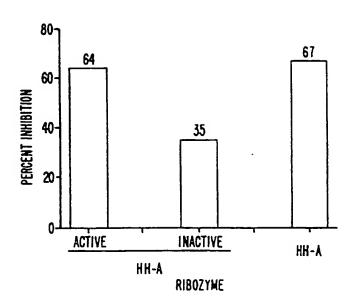


FIG. 66. SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

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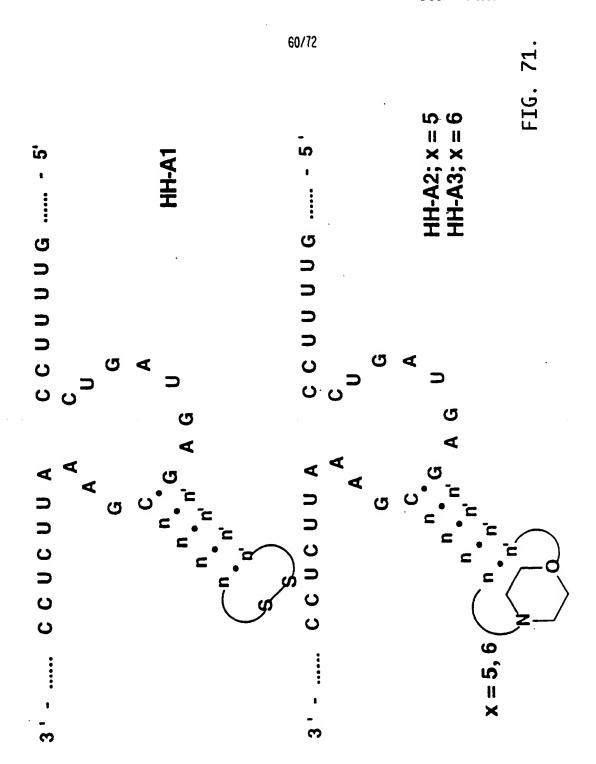
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NOTE: (CH₂)_n refers to any linkage. In addition, X and Y can be interchanged.

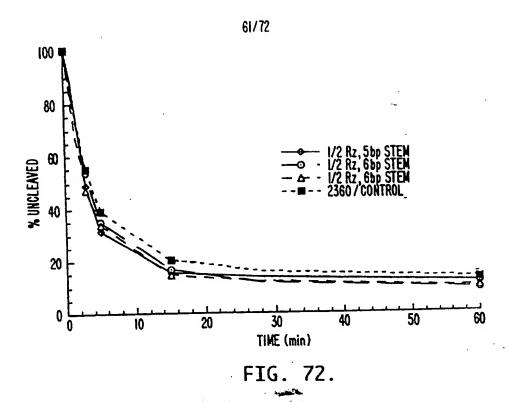
 $X = (CH_2)_nSH$, $Y = (CH_2)_nSH$ disulfide $X = (CH_2)_nNHR$, Y = ribose morpholino $X = (CH_2)_nNHR$, $Y = CO_2H$ amide $X = (CH_2)_nX$, $Y = (CH_2)_nOH$ ether, X = halogen $X = (CH_2)_nNHR$, Y = CHO amine $X = (CH_2)_nPPh_3$, Y = CHO double bond $X = (CH_2)_nPPh_3$, Y = CHO sulfonamide $X = (CH_2)_nNHR$, $Y = (CH_2)_nSO_2CI$ sulfonamide $X = (CH_2)_nOH$, $Y = CO_2H$ ester $X = (CH_2)_nOH$, $Y = CO_2H$ thioether, X = halogen $X = (CH_2)_nCOX$, $Y = (CH_2)_nCH$ carbonate, X = halogen

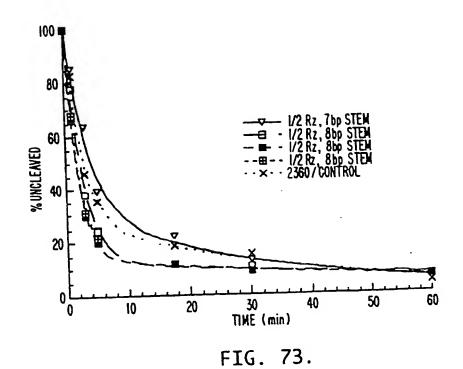
FIG. 69.

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)





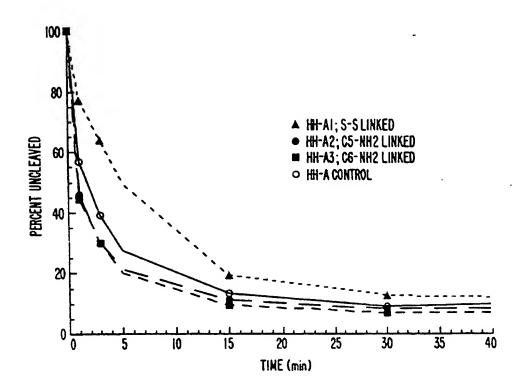
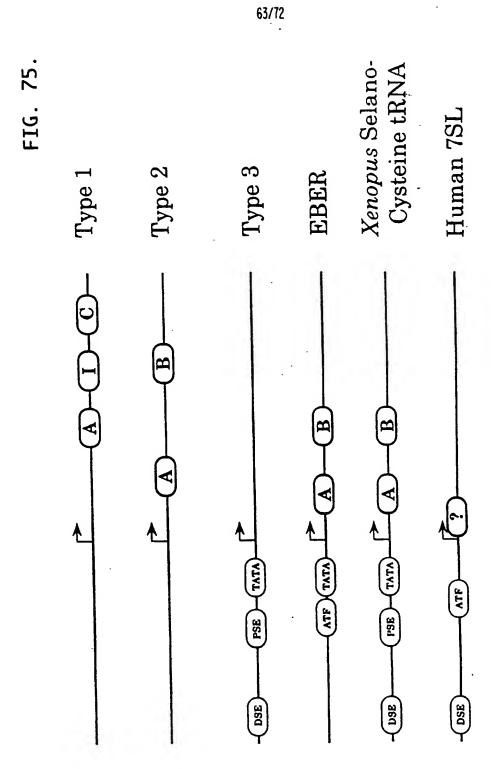
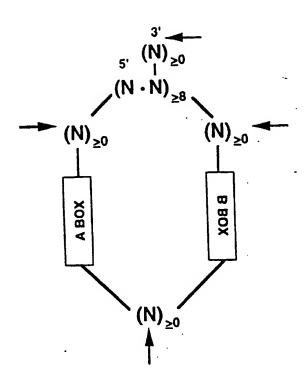


FIG. 74.



SUBSTITUTE SHEET (RULE 26)



A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

- = Indicates covalent linkage

= Indicates sites at which desired RNAs can be cloned

FIG. 76.

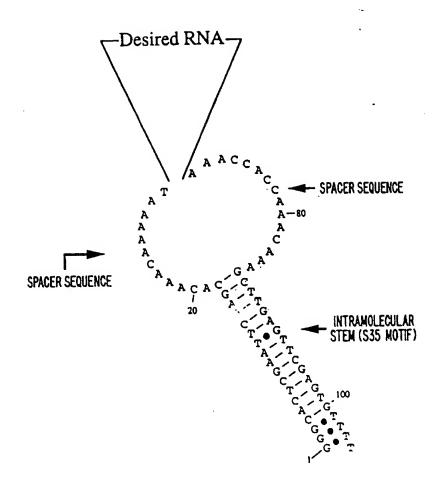
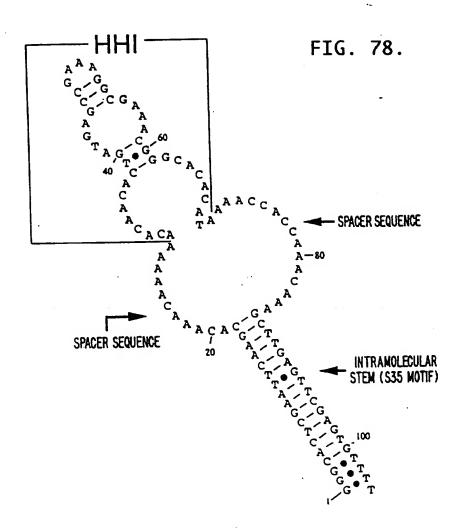
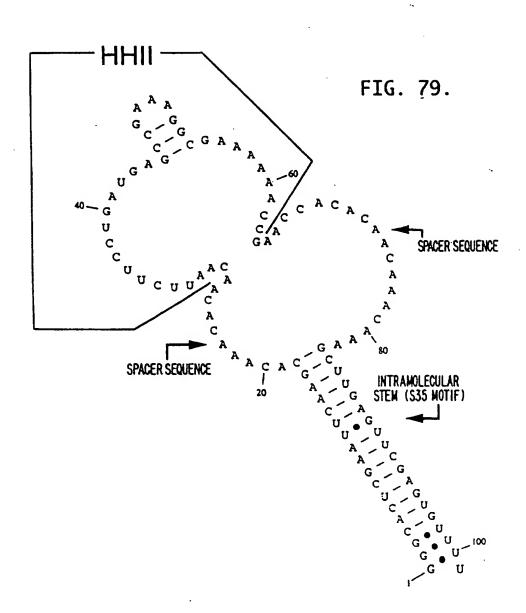
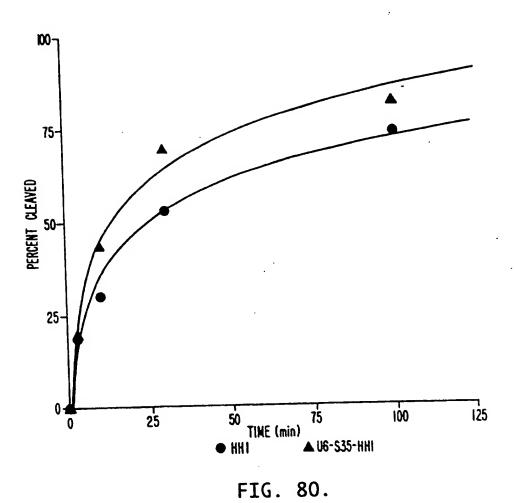


FIG. 77.







SUBSTITUTE SHEET (RULE 26)

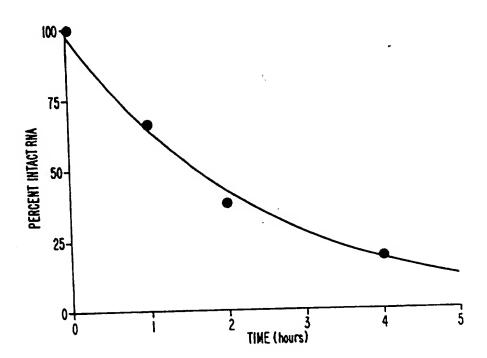
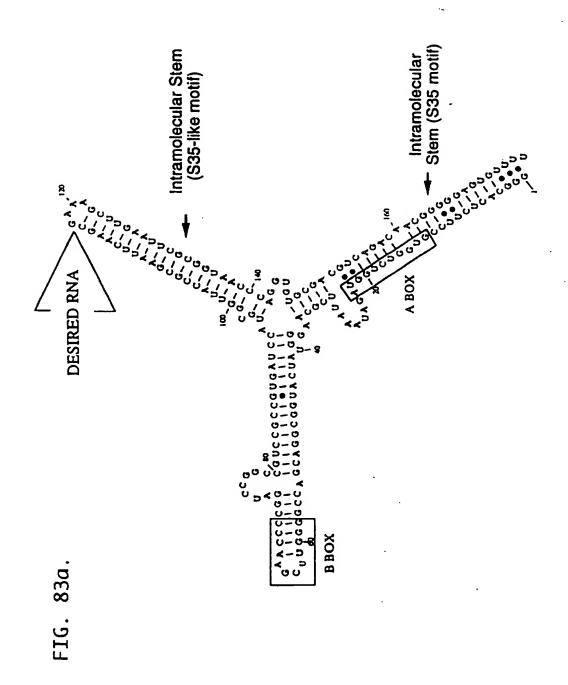


FIG. 81.

```
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                                                     FIG. 82.
                              C-G
A-U
SO G-C
G-C
C-G
Apical Stem-loop
                                A U-A AG CUGUGCG G-C AG G-C AG CUGUGCG
                                                    Central Domain
                                  C-G
G-C 130
                                  C-G
                                  G-C 140
                                   U-A
                                   G-C
                                   C-G
                                   C-G
      Terminal Stem
                                   U•G
                                10 Ŭ•G
                                   C-G
                                   U-A
                                   C-G 150
                                      C
                                   G-C
                                   G•U
                   SUBSTITUTE SHEET (AULE 26)
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SUBSTITUTE SHEET (RULE 26)



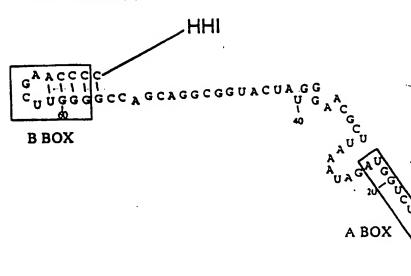
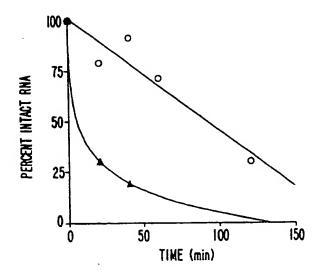


FIG. 83b.



▲ VAI-CHIMERA

• VAI-S35-CHIMERA

FIG. 84. SUBSTITUTE SHEET (RULE 26)